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EPIDEMIC AND ENDEMIC DIARRHEAL DISEASES OF THE INFANT

BY

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Conference Co-Chairmen: JAMES A. BAKER AND ERWIN NETER
Consulting Editor: ERWIN NETER

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* This series of papers is the result of a conference on *Epidemic and Endemic Diarrheal Diseases of the Infant* held by the New York Academy of Sciences, Section of Biology, December 8 and 9, 1955

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INTRODUCTORY REMARKS

By Hilary Koprowski
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It is not my function, in making these introductory remarks, to describe the dysfunction of the alimentary tract as a subject of this monograph. This will be done by Erwin Neter, who, jointly with James A. Baker, planned the conference upon which this publication is based. One glance at the table of contents of this volume will indicate that what these two eminent scientists have accomplished merits great admiration.

The alternate problems of diarrhea and constipation are probably more important to mankind than all other ailments combined. These generalized disorders profoundly affect the well-being not only of man, but also of domestic animals, wild animals and, possibly, every other living creature.

We know that dysfunction of the alimentary tract is not an entity, but represents merely a variety of conditions of different etiology. Discussion of all these conditions would take weeks, and it is wise to limit the scope of these deliberations to one specific aspect of the disorders; namely, the diarrheas of the infant.

The publications of the Academy deal with definite topics in order to combat one of the ills from which science suffers; namely, the defect characterized by Pickering[1] as "fragmentation of knowledge." These pages provide a unique opportunity to review a synthesis of thoughts rather than "canalization. . . into a restricted compass."[1]

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PREFACE

By Erwin Neter

Children's Hospital and University of Buffalo, School of Medicine, Buffalo, N. Y.

Extraordinary advances have been made in our knowledge of the etiology, pathogenesis, diagnosis, specific therapy, and prophylaxis of epidemic and endemic diarrheal diseases of infants. This conference affords an unusual opportunity to virologists and bacteriologists, to veterinarians and physicians alike to present and to evaluate critically the present status of this topic and to appraise the need for future research. Two subject matters stand out in these presentations: one, dealing with the various microorganisms acting as pathogens, and the other, concerned with the particular susceptibility of the young that differs so markedly from that of the older animal or human being. As a guidepost to our deliberations, may I quote one of the great pioneers in the field of microbiology, Louis Pasteur. He said: "Keep your early enthusiasm, dear collaborators, but let it ever be regulated by rigorous examinations and tests. Never advance anything that cannot be proved in a simple and decisive fashion. Worship the spirit of criticism. If reduced to itself, it is not an awakener of ideas or a stimulant to great things, but, without it, everything is fallible; it always has the last word."

DIARRHEAL DISEASES OF MAN: A HISTORICAL REVIEW AND GLOBAL APPRAISAL*

By Albert V. Hardy
Florida State Board of Health, Jacksonville, Fla.

Introduction

Attention will be limited to three aspects of this very broad subject. The magnitude of the current problem of mortality from diarrheal diseases will be examined in the light of past and present experience in the United States. Next, there will be an attempt to assess the limitations of present knowledge concerning etiology. Last, the adequacy of past and present programs of control will be observed.

Mortality from Diarrheal Diseases

Deaths from diarrheal diseases in present mortality statistics are classified in part as diseases of the digestive system, in part as specific infectious diseases, and in part as diseases of the newborn. Most are recorded as due to diarrhea and enteritis or, as it is now called in official records, gastroenteritis and colitis. Deaths reported as due to dysentery—usually type undetermined—and salmonellosis are found with the infectious diseases. Recently there was the further separation of epidemic diarrhea of the newborn. In general, the acute diarrheal diseases do not often receive an etiological diagnosis. Terminology varies by area, so the same clinical entity may be reported as diarrhea and enteritis in one area and dysentery, type undetermined, in another. Hence, in this review, unless otherwise specified, the designation "diarrheal diseases" will include deaths due to diarrhea and enteritis, and to dysentery of any type.

The accuracy of mortality data in all countries is determined by the reliability of the original diagnoses. It must be acknowledged that diagnoses of the diarrheal diseases are chiefly symptomatic, and that mortality statistics can provide evidence only on a group of disorders in which the outstanding clinical manifestation is diarrhea. In some countries from which vital statistics are available, it is evident that a significant proportion of the births and deaths of infants goes unrecorded. The commonest defect of the statistical data in places where public health is not well developed, however, is the reporting of deaths with either an ill-defined diagnosis or none at all. This was a problem of earlier decades in the United States when, in some predominantly rural states, many of the reported infant deaths had no usable diagnosis. In a rapidly developing progressive South American country, during the years from 1930 to the present, from 40 to 60 per cent of the death certificates had no medical diagnoses. This represents the approximate proportion of deaths without medical attention during the terminal illness. In such areas, the mortality rates for specific diseases, as computed, will be only a fraction of the presumed total

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* Data presented here were assembled, in part, for use in the consideration of the "Control of Infant Diarrheas in the Light of Recent Scientific Progress" at the Fourteenth Conference of the Pan American Sanitary Bureau in Santiago, Chile, in October 1954. Further statistical data were made available through Ruth Puffer, Chief, Statistical Section of the Regional Office of the World Health Organization for the Americas.

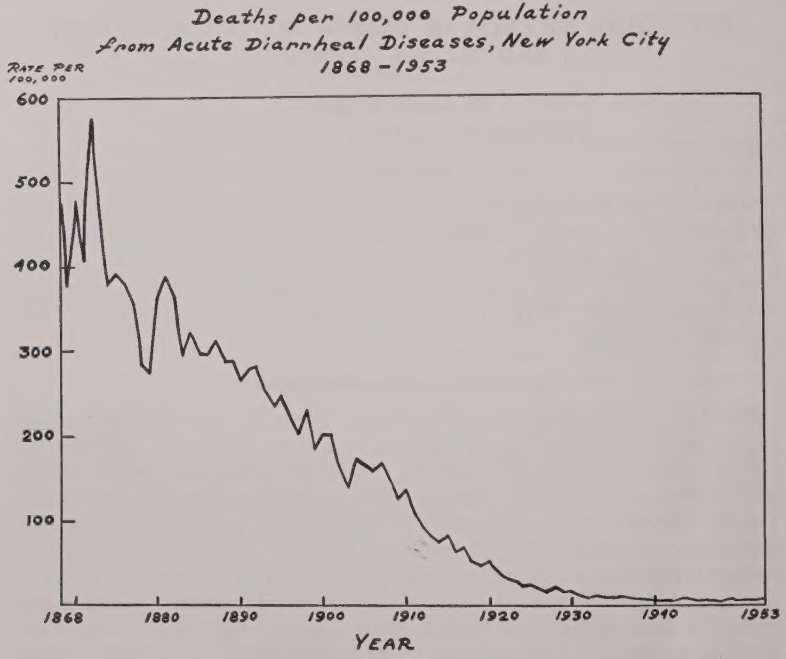

FIGURE 1

rate. Discrepancies will be marked for conditions primarily involving infants. Thus, the mortality data as given here will tend to understate the magnitude of the problem of diarrheal diseases for those countries with inadequate medical services and with poor communications.

While acknowledging inadequacies and inaccuracies in records of mortality, these are still the best index available of past and present incidence of diarrheal diseases. Data for the City of New York, available from 1868, on mortality from diarrheal diseases is presented in FIGURE 1 by year. Earliest records reveal in excess of 400 and reaching as high as 572 deaths per 100,000 population per annum. In the 25 years following 1875, the mortality declined from the 400 to the 200 level. In the first decade of the present century, there was a further fall from 200 to 100. A rapid and progressive reduction brought the mortality from 100 to 10 in the next 20 years. Since 1930 there has been a slow downward drop toward the present level of 2 deaths per 100,000 population from all diarrheal diseases.

Mortality rates are available for few cities in the United States for years earlier than 1900. Available data indicate that other localities had a problem similar to that in New York City. In most, during the period from 1900 to 1930, there was a rapid decline from excessively high to moderately low mortality from these diseases. In general, rates for states as a whole were appreciably lower than in cities, since at that time the mortality from diarrheal diseases was generally less in rural than in urban populations.

At the turn of the century, mortality from diarrheal diseases was high everywhere in the United States. In some states, there was an early and rapid decline, while in others there was a delay both in time and rate of fall. Thus, in later years the variation in mortality was great, ranging from high in a few to very low in many. This wide distribution is illustrated by data for 1936. In Puerto Rico, recorded mortality from diarrheal diseases was 480 per 100,000 population. In Arizona and New Mexico, the rates were approximately 100. In the remaining states, they varied from just under 50 to a low of 4.

Furthermore, in individual states there has been wide variation in different areas and in different population groups. Within the same geographical area, some segments of the population have little or no enteric disease, while in others the mortality from these causes may equal that which prevailed generally 50 years ago. In Hidalgo County, Texas, for example, from 1942 to 1945, the mortality rate for diarrheal diseases in permanent residents living under comfortable economic circumstances was 12 per 100,000 population while it was approximately 400 in the families of recent laborer immigrants. Similar wide variations have been evident more recently in California on comparing the general population with the families of migratory laborers. In the United States, a major remaining problem is to provide effective control measures for diarrheal diseases in such scattered foci of high incidence.

The magnitude of the current problem in the countries of the Americas is indicated by the data in TABLE 1. Mortality rates for diarrheal diseases are comparatively low only in the United States and Canada. In 3 of the countries, recorded rates are between 20 and 50 deaths from diarrheal disease per

TABLE 1

DEATHS IN 1952 PER 100,000 POPULATION FROM DIARRHEAL DISEASES IN COUNTRIES OF THE AMERICAS

Country	Deaths per 100,000 population
U. S. A.	6.2
Canada	9.6
Paraguay	21.0
Argentina	34.2
Uruguay	40.6
Chile	52.5
Peru	56.1
Panama	67.5
Bolivia	78.4
Venezuela	103.7
Dominican Republic	134.2
Colombia	135.5
Honduras	147.0*
Nicaragua	150.8
Costa Rica	176.8
Mexico	272.8
El Salvador	342.0
Guatemala	357.0

* Includes "Intestinal Infection."

100,000 population. In 4 others, rates are between 50 and 100; in 6 they are between 100 and 200; in 1 between 200 and 300; and in 2 over 300. The 6 countries with the highest rates are those south of the Rio Grande and north of Panama. Those with the lower rates are the countries with more temperate climates in the far south of South America.

Only limited mortality data are available from Brazil, the second most populous country in the Americas. In São Paulo, Brazil, the rapidly growing modern industrial city of the south, there were 124 deaths per 100,000 population from diarrhea and enteritis in 1952. In the same year, in other urban centers from which limited data were available, the total reported deaths from diseases of the digestive system were 306 per 100,000 population, which suggests a rate of about 250 deaths from diarrheal diseases. Obviously, in this vast country of about 60 million population, there is currently a high mortality from acute diarrheal diseases.

The 5 major causes of death in 1952 have been reported by 16 of the countries of the Americas other than the United States and Canada. In 9, the the diarrheal diseases were the major cause of death, and in 3 others it was second in order of importance. In 4 countries only, these diseases did not appear as one of the 5 major causes of death. On the basis of this evidence, the prevention of diarrheal disease must be acknowledged as the major public health problem in most of the countries of the Americas.

Though the current problem is of great magnitude, there is evidence of a gratifying downward trend in mortality, at least in Mexico. The data in FIGURE 2 are taken from the studies of Olarte, Aldama, and Varela.[1] In Mexico City, 25 years ago, the mortality from diarrhea and enteritis varied from 500 to more than 700 deaths per 100,000 population, rates even above those for New York City prior to 1875. The rate of decline in the Republic of Mexico from 1931 to 1950, however, closely paralleled the rapid decline in New York City from 1868 and 1887. At the end of these two decades, in New York in 1887 and in Mexico in 1950, the mortality was approximately the same, about 300 deaths per 100,000 population. The difference is one of time.

Turning from the Americas to other populous areas of the world, there are very limited data from Africa or Asia. The problem of diarrheal diseases has been studied in Cairo, Egypt, by the United States Navy Medical Research Unit No. 3, and the high prevalence of enteric infections has been clearly demonstrated.[2] For the country as a whole, mortality data are available only from localities with a "Health Bureau." In 1952, in these regions, the reported mortality from diarrhea and enteritis was 766 deaths per 100,000 population.

For Asia, data are available from Japan, which has rather well-developed medical and health services. The mortality rate from diarrheal diseases in that country in 1952 was 67 per 100,000 population. Ceylon reported findings also, with a total of 54 deaths from diarrheal diseases per 100,000 population. The reliability of these data is unknown, though De Silva,[3] of Ceylon, calls attention to an increasing mortality in children under two years for the period from 1946 to 1952, a condition that suggests an improvement in previously incomplete reporting of causes of death.

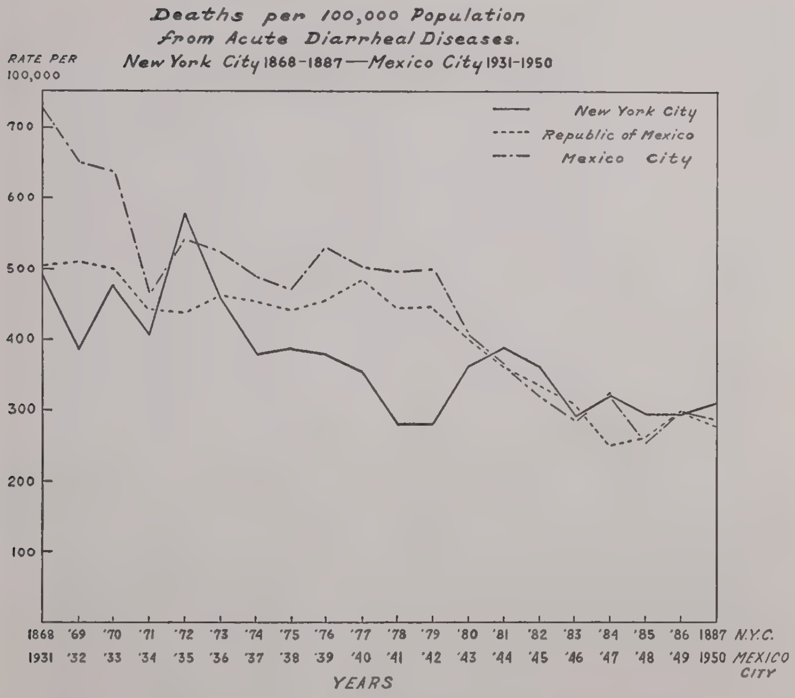

FIGURE 2

The extent of the problem may be examined further by determining which countries have a relatively low mortality from diarrheal diseases. These observations are probably quite dependable, due to the usual association of adequate medical and health services and complete reporting of causes of death and low mortality from infectious diseases. The mortality from diarrheal diseases in 1952 was less than 10 per 100,000 population in only a few countries. These included the United States, Canada, Australia, New Zealand, and the northwestern countries of Europe. Portugal, to the south, which was not included, reports a mortality of 130 for diarrheal diseases. The rate for Spain is lower, but still high. These countries of low mortality, together, have a population of approximately 300 million, or only one eighth of the total world population. Thus most of the world's children must still live through a substantial risk of acquiring a fatal diarrheal disease.

Illustrative age-specific death rates are given in TABLE 2. These provide full justification for emphasis on the control of diarrheal disease in infants. When there were 200 deaths per 100,000 total population in New York City, there were 4,496 infant deaths per 100,000 population under 1 year of age. It is observed, in contrast, that the death rate in older children and young adults

TABLE 2
AGE-SPECIFIC DEATH RATES (PER 100,000 POPULATION) FOR DIARRHEAL DISEASES BY AREA AND YEAR

Age	New York			Costa Rica 1953	Colombia 1948	Venezuela 1951
	1901	1910	1920			
Total	200	136	52	155	144	85
0–1	4496	3806	1796	2642	2302	1517
1–4	470	295	120	326	363	193
5–9	23	8	5	24	28	14
10–14	12	4	1	2	10	3
15–24	6	1	1	4	12	2
25–34	14	5	2	3	15	3
35–44	16	5	3	4	26	4
45–54	30	16	5	14	23	11
55–64	100	44	11	47	55	17
65–74	249	125	29	165	141	37
75 and over	529	329	124	313	379	71

was low, but in individuals of more than 75 years, there was again a substantial rise to a maximum of about one tenth the rate for infants. An examination of age-specific rates further shows that in the decline of mortality, there was an earlier and more rapid fall in the mortality in older children and adults as compared with a slow and delayed decline in infants. The current age distribution of deaths in three countries to the south are closely similar to those for earlier years in New York. The need for control in infants is more urgent and attaining prevention is more difficult.

Thus, it may be concluded that the acute diarrheal diseases either have been or are now the major disease problem of infants and children in all countries. The present variations in mortality from these causes in different areas are related chiefly to differences in the rapidity with which these preventable diseases have been controlled. In some cities and countries, present mortality from these causes is less than 1 per cent of that of earlier years. In others, for varying reasons, the downward trend has scarcely begun. It may be assumed that in populous and economically backward countries with inadequate medical and health services the mortality rates from diarrheal diseases, though unavailable, are still at a maximum level. We have before us for consideration, therefore, one of the world's oldest health problems which remains today as the major killer of infants and children in many lands.

Etiology

Historically, the outstanding feature in the accumulation of knowledge of the etiology of diarrheal diseases of infants and children has been the wide differences in opinion and in teaching. This can mean only that evidence was variable, insecure, or lacking. This, in turn, was partly related to the fact that when or where diarrheal diseases were prevalent, ordinarily facilities for their study were lacking. As medical and social conditions improved, opportunities for study became available, but the need and urge for study declined with the reduction in the incidence of disease. This inverse relationship of need and opportunity largely accounts for the fact that studies of diarrheal diseases in areas of high prevalence are limited and wholly inadequate. These diseases, which are currently the major cause of death in about half of the countries of the Americas and in many other parts of the world, obviously have not received the attention of medical scientists which their relative importance warrants.

There is ample evidence that valid findings in the etiological study of diarrheal diseases differ by area, age, character of illness, and by season. Flexner and Holt,[4] however, in their surprisingly effective studies in New York City in 1903, established a major observation in the etiology of diarrheal disease. Since then, it has been adequately confirmed that, where endemic diarrheal disease of infants and children is prevalent and with a high summer incidence, the commonest single cause is *Shigella*. Consider, for example, the recent observations of Floyd,[2] and of Higgins, Floyd, and Kader.[5] Studying representative children in an Egyptian village, they found first that virtually every child acquired *Shigella* infection, with or without symptoms, one or more times during the year. As high as 25 per cent of these representative children were found infected with *Shigella* by a single culture examination during the summer. These investigators found also a high correlation between the occurrence of diarrheal disease and the isolation of *Shigella*. Repeatedly, and in many countries, it has been found that where diarrheal disease is a common and serious disorder of children, *Shigella* infection is prevalent. This important role of *Shigella* in the etiology of diarrheal disease is given particular emphasis, since shigellosis is not scheduled for discussion in this monograph.

Salmonellosis, however, will receive subsequent consideration. At this time, attention is called only to the marked variation in the relative prevalence of *Shigella* and *Salmonella*. Commonly, many more cases are found due to the former than to the latter. For reasons not clearly established, however, *Salmonella* has been found to be a more important etiological agent in some areas than *Shigella*.

While the etiological role of these two organisms in diarrheal disease is established, it is nevertheless highly significant that in all studies there is a variable but substantial percentage of cases from which neither *Shigella* nor *Salmonella* can be isolated. Moreover, the percentage of "negative" cases is particularly high in young infants and, in the milder, less acute illnesses of older infants and young children. Indeed, in many areas with modern living facilities, shigellosis as a significant problem has been eradicated and salmonellosis is comparatively rare. Problems of diarrheal disease still persist, however, even though, by comparison, the mortality is gratifyingly low. The importance of etiological studies of these persisting illnesses is readily acknowledged.

Concerning other causes, there is wide agreement that *Endamoeba histolytica* is not an important cause of diarrheal disease of the young.

The role of bacterial agents other than *Shigella* and *Salmonella*, and particularly the enteropathogenic *Escherichia coli* will receive detailed attention in this monograph. The latter has been detected largely in residents of communities with modern sanitation. Is it possible that the control of dissemination of fecal pollution may be reducing immunity to *E. coli* infections so that the young infant lacking protection acquired from the mother is susceptible? This question is introduced to stress the importance of critical studies of the etiological role of enteropathogenic *E. coli* in countries with poor sanitary facilities and a high incidence of diarrheal disease.

The area of the unknown in the etiology of diarrheal diseases presumably will be reduced by the rapidly expanding studies of enteric viruses.

The detection of known or probable etiological agents will not be the complete answer to the pathogenesis of diarrheal disease. For example, future studies must establish whether viral agents may act alone, or in association with bacteria or other agents, to cause diarrheal disease in humans. The role of nutritional status per se and its significance in modifying susceptibility to enteric infection is highly important and must be clarified. The study of the interrelationship of these varying factors may well prove to be the most complex, and the most important problem in the investigation of the etiology of the diarrheal diseases.

In areas where facilities for study are accessible, there are broad gaps in the knowledge of the etiology of diarrheal diseases. In areas of high prevalence, there is very little secure evidence as to the various causes of these diseases. Particularly in these latter regions, a broad plan of study needs to be carried forward vigorously—as is appropriate for diseases which are a major cause of death.

Control

Available mortality data indicate that acute diarrheal diseases are a chief cause of death in many countries. This position as a major killer of mankind has been shared with malaria, tuberculosis and, periodically, with varying epidemic diseases. The malariologist could describe here the highly effective specific methods that have been used and are being applied with the hope of eventually eradicating malaria. This active campaign of magnificent proportions is a glowing example of successful public-health practices. Those concerned with tuberculosis can record the less dramatic but increasingly effective campaign against that disease. For the epidemic infections, there are the differing stories of the specific measures used to eliminate previous scourges—smallpox, diphtheria, typhoid, typhus, and yellow fever, to mention but a few. But what may be said for the means used to control the diarrheal diseases? There has not been and there is not now a generally accepted and widely applied program directed specifically at the control of these predominantly infectious disorders.

There appear to have been various reasons for this lack of a concentration of public-health attention specifically on the diarrheal disorders. Obviously, not one disease but a group of etiologically distinct entities had to be considered. This imposed difficulties in planning control. The role of defective feeding in these disorders was so emphasized in the past that control of diarrhea was regarded as a problem to be solved chiefly through attention to infant feeding. Even when these illnesses were considered as predominantly infectious diseases, there were no explosive epidemics to focus attention on the magnitude of the problem. Deaths from typhoid fever were relatively few by comparison, but startling outbreaks of that disease commanded action. The prevention of typhoid fever was accepted as a major public-health problem, and it was generally assumed that measures of community sanitation effective in preventing that enteric infection would have equal value in the control of diarrheal diseases. It was strikingly clear in some cities, notably Philadelphia and Pittsburgh, Pa., that this assumption was unwarranted. This is understandable, now, from the more adequate knowledge of the epidemiology of the diarrheal diseases. We do not recognize variations in the value of human life by age, but one cannot escape the opinion that there was less concern for the diarrheal diseases since they were a hazard chiefly to infants. Deaths of the young, while unfortunate, were considered inevitable. Whatever the causes, there has not been and there is not now any specific activity for the control of diarrheal diseases that compares with the extensive programs employed in attacking the other major killers of mankind. The world-wide importance of diarrheal diseases as a cause of mortality urgently demands more attention to this complex problem.

There is the record of history that the diarrheal diseases are preventable. Prevention may be attained slowly, predominantly through the improvement of living standards, as apparently was the case in the United States, but a rapid decline in mortality and morbidity undoubtedly could be attained through acknowledging the relative magnitude of the problem and attacking it from multiple directions with appropriate force.

Diarrheal diseases in the United States have declined to a remarkable degree but they are a continuing current problem, calling for the interest of investigators, administrative health officers, and clinicians. For the other countries of the Americas, the prevention of deaths from diarrheal diseases must be acknowledged as a major health problem, urgently calling for more attention than it has received in the past. The evolution of effective means for the rapid control of diarrheal diseases in these areas of high prevalence may be our best contribution to other countries with similar needs. The practical public-health control of diarrheal diseases has not been solved satisfactorily. This problem stands challengingly before us.

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DIARRHEAL DISEASES OF ANIMALS: AN APPRAISAL

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Diarrhea is a symptom of many diseases. In some cases it is the principal symptom, the others being secondary. In many other instances the diarrhea is definitely secondary to primary processes occurring elsewhere than in the alimentary tract. In the first instance, the cause must be sought in the alimentary canal. It may be the result of inflammatory changes induced by plant or mineral poisons, of the irritation caused by the presence of animal parasites, or of enteric infections with protozoa, bacteria, or viruses. Transitory diarrheas are common in animals following sudden changes of the diet as, for example, when herbivorous animals are turned out to pasture in the spring. In the second category, a long list of generalized infections of animals in which diarrhea is a prominent symptom might be given. A few examples are rinderpest in cattle, cholera in swine, Rift Valley fever in sheep, distemper in dogs, panleucopenia in cats, and psittacosis in birds.

The agents that cause diarrheal diseases in animals fall into the same groups as those that are active in man with a single important exception, the coccidia. Coccidiosis generally takes the form of intestinal infections. Many of these are severe and highly fatal, particularly in young animals. We find important diseases caused by coccidia in all species of domesticated animals, with the exception of the horse. There are, of course, no important coccidial infections in man.

Animals are not as fastidious in their eating habits as man, and very often they are kept under conditions that do not permit them to be as fastidious as they would be if they were leading a free existence. When animals are kept in close confinement with many others of their kind and are compelled to take their food from the ground, they are frequently subject to massive exposure to many enteric infections derived from their herd or flock mates.

For the most part, our domesticated animals are raised for economic reasons only and there is little sentiment attached to the question of whether they live or die. Because of the high cost of labor, animal owners frequently conclude that it is more economical for them to accept moderate losses from enteric diseases than it is to take the precautions necessary to prevent them. The economics of the livestock industry is geared for certain disease losses. You and I pay for them when we buy meat, eggs, and other foods of animal origin. The livestock man pays for them only when the losses exceed the usual amount. If city dwellers only realized this, they might change their widely held idea that animal disease is a matter only for farmers to worry about. The practice of veterinary medicine is very much like the practice of human medicine, since the same principles and remedies are used. The greatest difference lies in the fact that animal life is not valued as highly as human life. Many surgeons collect fat fees from patients that are not worth 15 cents to society, whereas such uneconomic procedures have no place in veterinary practice, except to a small degree when pet animals are the patients.

The losses among animals from diarrheal diseases are very largely among the very young. Udall[11] in the United States and Jordan[5] in England claim that calf losses from a common enteric ailment amount to about 20 per cent. In small herds these losses usually are smaller but, in many large herds, they often are much greater. Many young pigs are lost annually from a virus-induced diarrhea. Puppies, kittens, and lambs suffer from highly fatal enteric infections. I have already mentioned the coccidial infections which cause severe losses in poultry, lambs, and calves, and lesser losses in swine, dogs, and cats. Pullorum disease has now been brought under fairly good control in this country, but this disease formerly took a heavy toll in young chicks and poults. Paratuberculosis, or Johne's disease, in cattle is an enteric infection that is becoming of increasing importance in the United States. This is an infection with a specific acid-fast organism manifested by profuse and constant or intermittent diarrhea that leads to the death of most of its victims. The disease is seen most often in animals that are two to three years of age. Infections, however, occur mostly in calfhood, the incubation period being long.

An interesting and important difference exists in the mechanism by which man and the domesticated animals protect their very young from the infective agents that they encounter immediately after birth. The human infant, as well as the newborn of the ape, the rabbit, the guinea pig, the rat, and the mouse are fully equipped at birth to resist many infections by virtue of the antibodies that they receive, passively, from their mothers while *in utero*. Ehrlich[2] probably was the first to show that antibodies could traverse the placenta from dam to fetus. It was much later that Little and Orcutt[7] showed that this was not the case in cattle. We now know that this is not true for many of the domesticated mammals.

It is a very surprising fact that the blood of the newborn calf contains neither antibodies nor even any globulins (Howe[3]). As it arrives in the world, the calf, therefore, lacks any antibody protection. Nature makes good this oversight, under usual circumstances, by packing these substances in high concentration in the colostrum or first milk of the dam. In the normal course of events, the newborn suckles within a few minutes after it is born. At that time its intestinal tract apparently has a degree of permeability that it very quickly loses, for the antibodies and globulins that are ingested shortly after birth may be found in the blood within an hour or two after ingestion. When the calf becomes several days old, antibodies so fed do not traverse the intestinal wall (Smith and Little[10]).

When all is going normally, the one system of protecting the newborn seems to work as well as the other. The system of placental transmission, however, is the more foolproof, since neither chance nor the deliberate machinations of man can prevent the infant from getting his ration. These factors can and do operate in the case of animals.

If the young calf is prevented from getting colostrum, or if the cow is milked almost to calving time, so that little colostrum has been allowed to collect, the calf's early career is likely to be stormy and its chances of surviving the first two weeks of life are not good. Within a few hours, or a day or two at most, such animals usually become greatly depressed, develop profuse diarrhea (scours), become comatose, and die. The whitish-yellow fecal discharges contain much mucus, sometimes streaks of blood, and often many gas bubbles. The odor is sourish and unpleasant but not putrefactive. Cultures show tremendous numbers of coliform bacteria and hardly anything else. The same organisms are found in the blood and all tissues after death, and usually may be recovered from the blood before death. The calf apparently is overwhelmed by a colon bacillus septicemia.

Jensen[4] long ago unearthed most of these facts and termed the disease a "colibacillosis". Many others have confirmed these findings and have studied the coliform organisms, endeavoring to learn whether or not they differed in any way from the same species found in normal animals. The earlier workers were unable to find any distinguishing characteristics of these "pathogenic" strains. When administered to adult cattle or to older calves by mouth in very large doses, these strains were no more damaging than strains derived from normal animals, that is, both were nondisease-producing. For these reasons many workers have been skeptical of these organisms as the true inciting cause of the disease.

The autopsy findings leave no room for doubting that the immediate cause of death in this disease is *Escherichia coli.* Since serological typing of this organism has been done, a better opportunity has been presented of learning whether the so-called pathogenic forms are really any different from others. More work to elucidate this difference needs to be done, but that reported by Wramby, cited by Lovell,[8] and that of Lovell himself, indicates that the types found in calf scours may also be found in apparently normal animals. Both investigators found, however, that certain types occurred much more commonly in cases of calf septicemia than others. Lovell concludes that "the etiological significance of strains of *Bacterium coli* in white scours of calves, as well as in some forms of diarrhea in babies, would seem to be established." Although several thousand cultures from calves were typed, neither Lovell nor Wramby found any of the types that have been found most frequently associated with diarrhea in infants. It appears probable, therefore, that the types affecting the two species are different.

It is not to be inferred from what has been said that calf scours occurs only in calves from which colostrum has been withheld. A clinically identical disease occurs in calves that have received colostrum. This disease is not often a serious problem in small herds but, in larger ones, in which many calves are born each year, it sometimes reaches disastrous proportions. Most of these herds have special calf barns that often are elaborately equipped. Such barns frequently become pesthouses, since the disease is continuously propagated in the new calves that are brought in at frequent intervals. The character of the disease is quite like that seen in calves that have been deprived of colostrum, and the tissues of the victims are flooded with the same types of coliform bacteria. This disease, however, is highly contagious, and many of the individuals that manage to weather its onslaught during the first few days of life develop pneumonia subsequently. Coliform bacteria are not present in the tissues of the pneumonic cases, but rather a miscellaneous collection including streptococci, pasteurella and, most frequently, *Corynebacterium pyogenes*.

Skepticism about the primary role of *E. coli* in scours has frequently been expressed in the past, and many investigators have speculated about the possibility of one or more viruses being implicated. The first evidence of such a situation was provided by Baker,[1] who isolated a virus in 1942 from the pneumonic lungs of calves sick from this disease. With this agent, which he was able to propagate for many generations in mice, Baker was able to produce both scours and pneumonia in normal calves. Moll[9] in 1952 was able to duplicate this work, and to show that the disease produced by experimental inoculation was naturally transmissible to other calves by pen contact. It therefore seems probable that the old familiar calf scours in colostrum-fed calves is a colibacillosis which occurs, as a rule, only in individuals that have their colostrum-induced resistance broken by other factors, of which the most important seem to be viruses.

Mention should be made here of the filterable agent that Light and Hodes[6] isolated from the stools of human infants suffering from diarrhea, that produces a severe hemorrhagic diarrhea in young calves. So far as I know, this agent has not been compared with any of those isolated from naturally infected calves, hence the relationship, if any, is not known. There never has been any evidence presented that served to relate the human and the calf diseases, in spite of their clinical similarity.

Since others in these pages will deal with specific enteric infections in animals, there is no occasion for me to deal with them here. The natural reservoir of the *Salmonella* is in animals, in which they occur both as inapparent infections and as the cause of diarrheas and severe septicemias. Certain clostridia cause highly fatal enteric diseases, especially in sheep. A number of different viral agents have been identified in connection with diarrheal diseases in animals—in fact, such diseases occur in practically all species. Finally, we have a number of diarrheal diseases, one being the so-called "winter dysentery" of cattle, in which the causative agent has not yet been identified with certainty. The role of other viruses, such as the psittacoid agent of York,[12] which seems to be fairly widespread in cattle, is still not fully elucidated. It seems highly probable that future investigations will bring to light still other agents that are carried blindly by animals, that is, not appearing until precipitating factors or stresses bring them to light in the form of diarrheas.

In every species of domesticated animals, we have serious and fatal diarrheal diseases. Comparisons of some of these ailments with those recognized in man are sure to be helpful. Such comparisons are made elsewhere in this monograph.

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HOST-MICROBE RELATIONSHIPS

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Host resistance is one of the two major factors controlling the infectious process, pathogenicity of the infecting organism being the other. This has been recognized since the early days of microbiology. It is recognized that certain general factors play important roles in host resistance. The age of the individual, his nutrition, the general state of his health, and other general factors all must be considered in defining host resistance. Also, the experience of the host with respect to previous exposures to antigens as a result of clinical or subclinical disease, or active immunization, may contribute materially to specific resistance or immunity. In this presentation, however, attention will be directed primarily to natural or innate host resistance.

Progress has been made in resolving the general factors of natural or innate host resistance into specific mechanisms. Our knowledge of the humoral factors, particularly the action of blood serum on microorganisms, has increased somewhat since it was first observed that heat-labile factors of serum acted on gram-negative bacteria, and that the heat-stable factors, the beta lysins, possessed antimicrobial properties against the gram-positive microorganisms.

The heat-labile or complement factors in blood serum have been extensively studied, particularly as they affect the hemolytic reactions.[1, 2] We are not too certain, however, as to the relationship of hemolytic complement and the heat-labile antimicrobial factors in serum, sometimes referred to as bactericidal complement. Possibly bactericidal complement requires a fifth component for its action. The discovery of properdin by Pillemer[3] may help in resolving the apparent difference between hemolytic and bactericidal complement.

Since there is currently a strong tendency to relate certain strains of *Escherichia coli* to infectious diarrhea of the newborn, it would seem appropriate to determine the action of the bactericidal serum complement components, including properdin, on the particular strains of *E. coli* thought to be the cause of this disease. If bactericidal complement does act on these strains, which may or may not be true, then the question arises as to whether these antimicrobial factors are present at birth.

Dancis and Kunz[4] have noted that the bacteriostatic activity of serum of both full-term and premature infants at birth was not too different from that of older children or adults, but that this activity tended to disappear within a few weeks and then to reappear after a year or so. This lack of bactericidal action of serum of infants older than two weeks against gram-negative organisms (*E. coli*, *Salmonella derby*, and *Neisseria catarrhalis*) was not related to hemolytic complement but appeared to be associated with a factor in serum gamma globulin.

Other body fluids, such as gastric, pancreatic, and intestinal secretions, may well be concerned in the resistance of the infant to the entrance of microorganisms by way of the gastrointestinal tract. Unfortunately, very little has been done to evaluate the role of such secretions in the host resistance of man at any age, let alone that of the infant.

It is currently recognized that bacterial ecology may play a role in host resistance. Disturbances in the bacterial flora of the intestinal tract produced by the administration of antibiotics may result in an overgrowth of potential pathogens as *Micrococcus pyogenes* or the yeasts usually present in small numbers in this area. Miller and his colleagues[5] have recently reported a tremendous decrease in the resistance of mice to *Salmonella* following a disturbance of the normal flora of the mice by preinoculation treatment with streptomycin. In recent studies in our laboratory, James Crawford has investigated the antagonism of bacteria isolated from the upper respiratory tract of man.[6] He found that of 33 strains of gram-positive cocci isolated from patients, 26 inhibited pneumococcus type I, 4 inhibited group A *Streptococcus* type 3, and 4 were able to prevent growth of *N. catarrhalis*. Other groups of bacteria from patients, such as gram-positive and gram-negative rods and *Neisseria*, also had some bacteriostatic effect on the test organisms investigated. If the normal flora does act as a defense mechanism, obviously the infant may be vulnerable to certain organisms that normally are held in check in the older host by the established flora.

Microorganisms in the inhaled air impinge on the mucus layer of the respiratory tract. They are either destroyed by the defense mechanisms in this tract or are moved by ciliary action to the oral pharyngeal area and swallowed. Thus airborne organisms, including the etiological agent of infant diarrhea present in hospitals, will reach the gastrointestinal tract unless they are destroyed in the upper respiratory tract. Bacterial ecology of the upper respiratory tract, as well as of the intestinal tract, therefore, must be considered in a study of host resistance to the causative agent of diarrhea of the newborn.

The phagocytic cells of the host are recognized as playing an important role in host resistance. There is general agreement as to the various types of cells that possess phagocytic properties. These, of course, include the circulating and fixed phagocytes. The first prerequisite for phagocytosis is contact between the phagocyte and the microorganism. Chemotaxis, or attraction of wandering phagocytic cells to bacteria, may be either positive or negative in sense, depending on the microorganism concerned. The physical structure of the medium in which the phagocytic cells and bacteria are found may be quite critical in determining whether or not phagocytosis occurs. Wood and his colleagues[7] have emphasized this phase of the problem under the title of "surface phagocytosis." The fixed phagocytes of the spleen, liver, bone marrow, *etc.*, may be aided in making contact with bacteria carried to them by the blood stream by virtue of a decreased rate of flow of blood through a large capillary bed. Also, it is possible that blood flow may actually be momentarily stopped in certain capillaries in the bed by a valvular control of these capillaries as suggested by Knisely.[8] If we admit the possibility of such a valvular mechanism, the question then arises, at what age in the developing infant does it begin to function?

Once contact between the pseudopod of the phagocyte and microorganism is made, phagocytosis may or may not occur, depending on the presence in the serum of normal opsonins, immune opsonins, or the nature of the phagocyte, the strain of the microorganism, and other factors such as an adequate ascorbic acid level in the phagocytic cell.[9, 10] Something, but not enough, is known of the comparative physical and chemical properties of the phagocyte and the microbe as affecting this important defense mechanism. It is confusing to realize that both phagocyte and microorganism carry an over-all negative charge.[11] Possibly if we knew more of the dynamics of this process, this anomaly might be resolved by finding that the portion of the pseudopod, or at least its advancing portion making contact with the microorganism, actually carried a positive electrostatic surface charge.

It has often been stated that the bacterial capsule interferes with phagocytosis, which may or may not be true, depending on the host of origin of the phagocyte and the strain of pathogen. The chemical nature of the capsule, as a factor determining whether or not phagocytosis will occur, appears to be more important than its physical structure.

Once phagocytosis has occurred, the pathogen must be destroyed if this process is to be an effective host-defense mechanism. For many years it has been realized that phagocytic cells varied markedly in their ability to destroy microorganisms. Whether or not the microbe is or is not destroyed depends on such factors as the host of origin of the phagocyte, the type of phagocytic cell, whether or not the host has been previously immunized, and the strain of microbe. The enzymatic mechanisms by which microbes are destroyed within phagocytic cells have not been clearly defined to date. Advances are being made, however, in the general enzymology of phagocytic cells. Various enzymes have been isolated from these cells,[12] their metabolisms have been studied to a limited degree,[13] and a few investigations on the effect of fractions of the pathogens on the metabolic mechanisms of leukocytes from resistant and susceptible hosts have been made. In our laboratory, some comparative studies of this nature have been done by Alonso,[14] who has found that the specific polysaccharide of type I pneumococcus stimulated anaerobic acid production by rat leukocytes about 10 times more than it stimulated the same metabolic activity of guinea pig leukocytes over a period of observation of 90 minutes. The guinea pig is many times more resistant to the pneumococcus type I than is the rat.

Since energy is required for all phases of the phagocytic process, Murayama,[15] in our laboratory, studied the effect of pneumococcus type I polysaccharide and the anthrax-immunizing antigen of Wright[16] on the adenosine triphosphatase (ATP'ase) activity of this enzyme isolated from rat and guinea pig leukocytes. These animals vary markedly and in an opposite sense in their susceptibility to the pneumococcus and *Bacillus anthracis*. Muryama found that the ATP'ase from the leukocytes had greater activity in the presence of these "virulence factors" if the enzyme came from a resistant animal, and was depressed if the enzyme source was a susceptible animal.

Since the ultimate conflict of the pathogen and the host is decided at a biochemical level, it would seem very desirable that more comparative biochemical investigations be made of host resistance mechanisms.

Bacon, Burrows, and Yates[17] have found that certain strains of *Salmonella* unable to synthesize para-aminobenzoic acid (PAB) would not produce infection in mice unless the level of available PAB in the mouse was artificially raised. This approach to a biochemical basis for host resistance emphasizes the possibility that host resistance may depend on the absence of a necessary growth factor for the parasite. Conversely, of course, the presence of an antimicrobial factor in the host may prevent growth of the parasite. Obviously, both approaches must be developed if an adequate understanding of host resistance is to be acquired.

The mechanism of disease production is an important consideration in natural host resistance. Unfortunately, very little is known of the actual mechanisms involved in the production of signs and symptoms of disease in most infectious diseases. Certainly, infant diarrhea is one such disease in which our knowledge of the pathogenesis is lacking. The difference in susceptibility of two infants equally exposed to the etiological agent of infant diarrhea may depend in part on the ability of the organism to survive and grow in the body and in part to the reaction of the infant to the biochemical factors elaborated by microbial growth.

Although there is little definitive knowledge as to how microorganisms produce disease, it is in order to review briefly what information we do have.

In some infections there is actual destruction of vital tissues of the body, as in poliomyelitis, syphilitic aortitis, and hepatitis. How these tissues are destroyed is not obvious, yet the fact that we know that they are gives some satisfaction in explaining the pathogenesis of these diseases.

In botulism it is now known that the toxin blocks the transmission of the nerve impulse across the neuromuscular junction. This gives an adequate explanation of the cardinal signs of this disease.

As a general proposition it may be said that the circulating blood is altered in an infectious disease. The changes that occur may impose an increased burden on the cardiovascular system, as seen in the intravascular clumping of red blood cells by bacterial polysaccharides which Youngner and Nungester observed in 1944[18] or in the related "sludging" of blood as reported by Kniseley.[19] The latter phenomenon is by no means limited to infectious disease. In either case a microscopic observation in arterioles of the circulating blood so affected impresses one with the increased cardiovascular effort required to keep the affected blood moving at an adequate rate.

The recent publication of Smith and his colleagues from England[20] on the pathogenesis of anthrax directs our attention to the effect of microbial infections on such subtle physiological reactions as shock, with its endocrinological implications. The introduction of ACTH and related compounds into medical practice has not only made possible the control of some of the signs and symptoms of the infectious process, but has suggested that disturbance of the patient's endocrine physiology may be an important factor in disease production in infections.

Miller[21] has reported that the endotoxin of the meningococcus interferes with glycogenesis by the liver. The current interest in electrolyte distribution between tissues and body fluids in shock and other clinical conditions may ultimately lead to a better understanding of the origin of signs and symptoms in infectious diseases. Certainly in infant diarrhea there is marked alteration in electrolyte balance as a result of the infectious process.

In summary, it must be assumed that host resistance to a given parasite is an algebraic summation of the various defense mechanisms of the host. In the future it may be possible to set up an equation with appropriate qualifying exponents to describe the total resistance of the host. This is currently done to a limited degree in describing the resistance of the immunized host with respect to circulating antibodies. Even here, this approach is not entirely adequate, since no measure is made of intracellular antibodies and since the natural or innate defense mechanisms are not adequately considered as an important component of the entire picture.

Biologists, as a class, are not sympathetic to the mathematical approach. Two reasons for this are apparent. First, much of our data is not quantitative and, second, we, as biologists, usually lack a mathematical background. As our sciences mature, both defects will be corrected. Hence, we can look forward to a more exact description of host defense mechanisms even on a mathematical basis.

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GENETICS AND NUTRITION OF THE HOST IN RELATION TO SUSCEPTIBILITY TO INFECTION

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The opening papers of this monograph have the theatrical assignment of erecting some of the scenery against which the action of the drama to follow will be appropriately set. They also serve as a background to remind us gently of the locale that brings together this varied cast, as its members successively appear. My background paper is a discussion of genetics and nutrition of the host in relation to susceptibility to infection. I think I must warn you that I shall use a large brush, and that, when finished, the piece will look its best, like most theatrical scenery, when viewed from a slight distance.

Our discussion, then, will involve two sciences and one phenomenon: the sciences of genetics and nutrition and the phenomenon of susceptibility to infection. Let us assume that we are fairly clear about the general character of the descriptions and predictions that form the content and the aims of the two sciences of genetics and nutrition. If these sciences are left somewhat blurred by so cavalier a treatment, it is only because, for the moment, I want to focus more sharply on the notions that swim in our heads when we are confronted with the concept of susceptibility to infection.

What is susceptibility to infection?

One definition might be "the capacity to respond to a microbial agent with the signs and symptoms labeled as disease." With increasing sophistication, we might broaden this definition to include those instances in which the pathogenic agent produced no overt disease, but was successfully entertained by the host. Historically, however, it is probably true that it was the signs and symptoms of disease that first aroused our interest. Let us accordingly use the former definition as a temporary touchstone.

Where shall we find the phenomenon of susceptibility?

Obviously, wherever we encounter disease, for the diseased host is, *ipso facto*, a susceptible host. In the natural history stage of such an inquiry we soon face the problem of categorization and, taking a clue from the taxonomists, we are able to distinguish susceptible species and insusceptible species in terms of any given specific pathogen. For example, H. Zinsser (1919), confining himself to some of the more important diseases of man, listed 24 different pathogens to which a wide variety of other animal species proved to be insusceptible. Familiarity with these interspecific differences in susceptibility is so widespread as to pass almost unremarked, yet—and this may be the reason—the basis for these important differences is very poorly understood. Before we avert our gaze from this troublesome state of affairs, it might be useful to sketch briefly what is certainly not an insight or understanding but what might be euphemistically described as an attitude toward this problem.

In describing these interspecies differences in susceptibility, which are of the all-or-none variety, I have continued to allude to the lack of susceptibility as insusceptibility and have avoided the use of the term "resistance." My reasons for this are purely semantic. Resistance is a Newtonian term with many connotations of an opposed force. As we shall see in the sequel, this word will be readmitted to our vocabulary in its logical role. At the moment, however, there does not appear to be any evidence that insusceptible species are insusceptible because they contain entities that might logically be construed as being mechanistically opposed to activities of the pathogen and that are absent in susceptible species.

To make this clearer, let us return to a scrutiny of the concept of susceptibility. We have seen that this capacity to respond is an attribute of some species and not of others. In a word, it is a biological characteristic and thus is subject, in an evolutionary sense, to the same laws that govern the transmission of other biological characteristics. This suggests that susceptibility has an hereditary basis, and the species differences would argue that this is indeed the case. There is a block, however, in the attempt to penetrate into the genetics of these interspecific differences, because the *modus operandi* of the geneticist, directed mating, is denied to us. This is due, of course, to the fact that interspecific matings produce no viable offspring. In those rare instances in which viable progeny are obtained, as in the horse-donkey cross, I have been unable to find any data as to the fate of possible parental differences in susceptibility as they might be reflected in the hybrid progeny. It should be mentioned that such susceptibility as appears in both parents, as does susceptibility to equine encephalitis in horse and donkey, for example, appears in the interspecific hybrid, the mule.

Having been helped but little by the operations of the science of genetics in our attempts to understand susceptibility in terms of interspecific differences, let us return to home base.

When we turn to experimental nutrition we find that we are no better off in our attempts to understand the bases of interspecific differences in susceptibility to infection. Although the data of comparative nutrition are somewhat inadequate, and a negative estimate is thereby limited, there is at present no basis for suggesting that any interspecific differences in susceptibility are explicable on the basis of known differences in nutritional requirements.

Let us now turn from interspecific differences to intraspecific differences in susceptibility, *i.e.*, differences *within* species. Here we find that the directed breeding operations of the geneticist are completely available, and we have a considerable amount of material from which to choose our discussion (Gowen, 1951). In fact, this material is rich and varied enough for us to make several generalizations. Thus, whenever the necessary energy of the investigator has been expended, it has been possible to demonstrate that inbreeding and selection can arrange stocks of originally susceptible animal hosts that are divergent now in their susceptibility to infection. The extent of this divergence is a function of the original genetic heterogeneity with which the operations began. The pursuit of a totally insusceptible stock within an originally susceptible species seems to be unattainable, although the goal can be approached. The reason for this limitation may rest in limitations of the original gene populations or, more probably, may be inherent in the physiological boundaries to the phenotypic expression of the selected character.

Another important contribution that genetic studies of susceptibility have made is that differences in susceptibility to one disease are independent of such differences in terms of a second disease (Webster, 1937). Susceptibility to one disease and a relative insusceptibility to another can be assembled in the same animal. This is an important point, for it destroys completely the notion that differences in susceptibility can be embraced by such vague and sweeping notions as robustness or vigor. This demonstration is important, too, in that it makes necessary the introduction into this discussion of the element of specificity of the pathogenic agent.

Although it is true that most of what has been learned about the genetic control of susceptibility has come from animal experimentation, it must not be forgotten that genetic influences operate in man as well. By means of identical-twin studies, it has been shown that genetic constitution plays a major role in susceptibility to tuberculosis, the correspondence between affected index cases and the monozygotic co-twin being of the order of 90 per cent. The correspondence between sibs and dizygotic co-twins was, by contrast, only 25 per cent (Kallman and Reisner, 1943). It is hard to imagine, without directed breeding, a more striking demonstration of the operation in the human population of heredity as a major determinant of endemic infectious disease.

A recognition of host genetic constitution as a determinant of susceptibility to infectious disease underlines, once again, the central role in biology of the science of genetics. It is unfortunately true, however, that the very structure of human society prohibits the application of the geneticist's operations, directed breeding, as a solution of the problems of infectious disease. In these matters, genetic solutions can be reached only by the long and tedious grinding of the evolutionary mill. In our impatience to deal with these matters in the here and now, it is understandable that we should turn elsewhere. In the space that remains, let us examine one such alternative—the effects of host nutrition on the problem of susceptibility.

This is not the time or the place in which to discuss in detail the voluminous literature on nutrition and infection. The general drift of these attempts to relate nutrition and infection, however, have had several implications that, I think, have a relevancy to the subject of these papers. I should now like to discuss two of these implications.

The first of these is that, for purposes of further analysis of the phenomena of susceptibility, it may prove helpful if we recognize that there are separate and distinct biological bases for susceptibility and for what is commonly regarded as its reciprocal—resistance. In other words, susceptibility and resistance are not different aspects of the same thing, but are separate and different things. The reasons for this distinction have become pressing as a result of the urge to form some coherent estimate of what appear to be conflicting experiences in nutritional experimentation related to the phenomena of infection.

The troubles began, I believe, with the tacit assumption that survival in infectious disease was always and necessarily linked with the adding to or enhancement of something in the infected host. This idea appears to have been generally accepted as a consequence of historical developments in the study of infectious disease, chiefly because of the great influence of immunology, which is rooted in the notion of the causal connection between the adventitious antibodies and survival. "Something added" means "something required"; so, with disarming consistency, the view was soon gained that only an abundant and optimum nutrition of the host could fill the seemingly logical demand. When we turn to the results of nutritional experimentation in the field of infectious diseases, however, we find that experience does not always conform to this simple view. The fact is that increased survivorship, or diminished severity of disease can be achieved by *withholding* certain items from the diet; the same end can be reached by *adding* other items (Schneider, 1951, 1953). Thus, the desired goal can be approached by diametrically opposed and nutritionally unambiguous manipulations: deficiency on the one hand and supplementation on the other. If we are to remain clearheaded about this, I think the time has now come in which it will be useful to regard the susceptibility and resistance of an animal as separate and distinct biological attributes in an absolute sense. Our confusion has arisen, I think, because these two opposed characteristics are simultaneously present in the host, and because we are aware only of the resultant of these two opposed forces. Any given resultant—survival, death, or the stages between—can be achieved in two different ways: by the enhancement of the one attribute or the diminution of the other. The unambiguous operations of nutrition, the acts of withholding or supplying, now form the valid base for deciding which of these alternative processes has taken place. In order to find firm ground on which to stand, we can construct operational definitions. Thus, if withholding a given nutrient decreases the effect of an infection, and supplying the nutrient increases the effect, I think it will be useful if we say that the nutrient specified is a susceptibility factor, and that what we have manipulated is an abstraction that we shall call "susceptibility." On the other hand, when experiment shows us that withholding a nutrient enhances the infection and supplying it diminishes the disease, then I think it will be useful if we state that this nutrient is a resistance factor, and what we have manipulated is the abstraction that we shall call "resistance." We have now formally, and for the first time in our analysis, admitted the word "resistance." We now have an operational definition, however, to help us recognize its face when we meet it on some dark and stormy night.

Let me be clear about this. I am under no illusion that the operational definitions I have proposed will change our common discourse on these affairs. As far as *describing* our experiences, I am certain we shall all continue to use "more resistant" and "less susceptible" as equivalent and interchangeable. I submit, when we do so, however, we shall continue at the level of common discourse, and never get beyond it. It is when we come to *analysis* that we shall find the operational definition useful, and our ability to distinguish between resistance and susceptibility more than a nicety, but, I think, a necessity.

Among several examples of a specific nutritional deficiency *reducing* the effects of an infection, and therefore legitimately considered as a manipulation of susceptibility, I shall choose one. This is the mouse-mouse adapted poliomyelitis virus model, and the nutritional entities are several of the essential amino acids. Single amino acid deficiencies of eight of these amino acids resulted in a reduction of the signs of viral disease as evidenced by an increase in the incubation time and a decrease in the number of animals paralyzed (Davies *et al.*, 1952). Here, by unambiguous deficiency, susceptibility has been lowered. More than that, the experiments clearly show that this effect was a host effect, for the virus successfully multiplied in the brains of these deficient animals and, indeed, in the instance of tryptophan deficiency, when virus levels in the nondeficient controls had declined, the tryptophan-deficient tissues still showed high levels of virus. Lack of disease was not for lack of virus. I should say that lack of disease was for lack of the host to respond, which, as we said at the outset, *is* susceptibility.

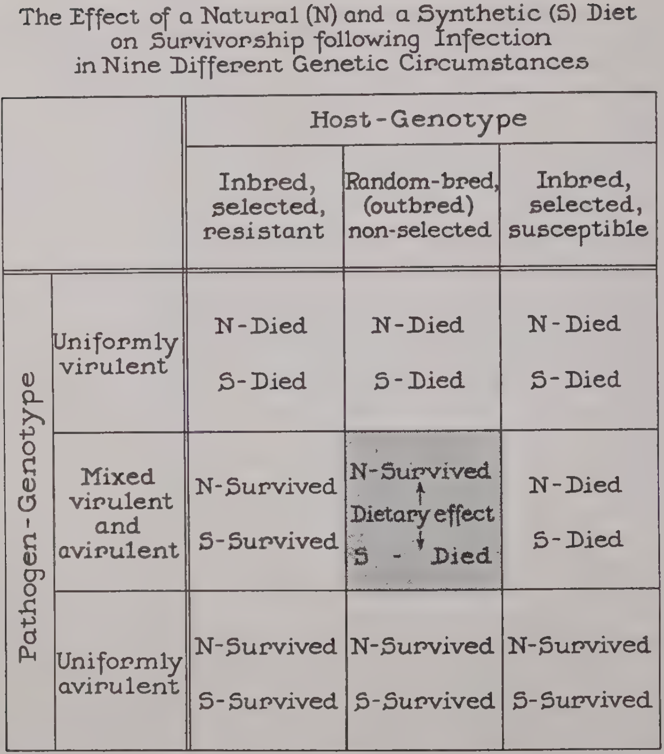
It is when we come to discuss nutritional effects on operationally defined resistance that we come to the second and last implication that I wish to discuss here. In the operational definition of resistance, we have demanded that the character be enhanced by supplementation and diminished by deficiency, *i.e.*, that supplementation improve survivorship and that deficiency reduce survivorship. Something that will do this, we have said, is a *real* resistance factor. Now, for some time, we have been investigating just such a factor in the mouse-salmonellosis model. It is apparently a new, unrecognized material, and we have succeeded in concentrating it over a millionfold from natural sources, such as wheat. This, however, is not what I want to emphasize here. Instead, I wish to underline an implication that we stumbled upon when we were initially concerned with what I shall call the biological dimensions of this model. For, in the early days of this investigation, we found that, in assembling an infection model, specifying the host and pathogen species, mouse and *Salmonella*, was not enough to ensure that one had a model that would respond to the nutritional influences we were seeking in the world of natural foodstuffs. Intraspecifically, both host and pathogen exhibited wide variation, and their genotypic dimensions were finally described, as in FIGURE 1.

The message of this figure is that, in this model, a nutrition experiment on natural resistance is made up of a suitably genetically heterogeneous host and a heterogeneous pathogen population. The figure also shows that host genetic differences similarly demand a heterogeneous pathogen population in order that phenotypic expression of these genetic host differences may be achieved.

All this tells us something new about intraspecific natural resistance. It is simply this: Natural resistance, although an attribute of the host, has its expression inextricably linked with what we might call the structure of the pathogen population.

Intraspecific natural resistance, then, is the successful solution of what is probably a metabolic problem precipitated by certain members of the pathogen population and solved by the presence and probable action of other members of the pathogen population. Natural resistance, though it is an attribute of the host, is not to be conceived as a barrier, an antibiotic, or opposed force. It should be considered a positive entity, biochemical in nature, which arranges for survivorship by assisting certain members of the pathogen population to come into adjustment with other members and the host, thereby permitting a silent coexistence of pathogen and host in which both survive as representatives of their species.

This notion of natural resistance conceived as a pathogen intrapopulational



The Effect of a Natural (N) and a Synthetic (S) Diet on Survivorship following Infection in Nine Different Genetic Circumstances

		Host-Genotype		
		Inbred, selected, resistant	Random-bred, (outbred) non-selected	Inbred, selected, susceptible
Pathogen-Genotype	Uniformly virulent	N-Died S-Died	N-Died S-Died	N-Died S-Died
	Mixed virulent and avirulent	N-Survived S-Survived	N-Survived ↑ Dietary effect ↓ S - Died	N-Died S-Died
	Uniformly avirulent	N-Survived S-Survived	N-Survived S-Survived	N-Survived S-Survived

FIGURE 1. From H. A. Schneider, in *Biological Foundations of Health Education*, Columbia University Press, New York, N. Y., 1950.

problem has many interesting consequences. By recognizing the importance of the frequency distribution of the numerous variants in the pathogen population, we can understand the general plasticity of such pathogenic agents as viruses at the time of their original isolation from nature and can be aware of the consequences of laboratory passage through uniform hosts upon the pathogens' more uniform behavior and increased "suitability" as items for study. What would it mean, for example, to use heterogeneous, unselected virus populations for a nutritional experiment? And what would be the consequences of attempting the same study with virus populations, after many laboratory passages, and of "uniform titer"? Similar questions suggest themselves whenever we reflect on the population structure of the pathogen used in our experiments, be they protozoan, viral, fungal, or bacterial.

Let us sum up, then. In one model at least, intraspecific natural resistance was shown to be influenced by nutrition only when the host was genetically heterogeneous and the pathogen population was heterogeneous in the sense that it contained an array of variation in terms of capacity to produce disease.

When the pathogen population was uniform, host difference, genetic or nutritional, had no effect. The outcome was decided by the nature of the uniform pathogen population. In a word, natural resistance disappeared. From this we can construct an operational definition of intraspecific natural resistance. The phenomenon has its basis in the host, but the solution for the situation has *its* basis in the intrapopulational problem of the pathogen. Thus the host and the pathogen population are inextricably woven into the conceptual fabric we know as intraspecific natural resistance.

In summary, let me remind you of the two things that, I think, genetic and nutritional experimentation has illuminated in the phenomenon of susceptibility:

(1) It is theoretically desirable and practically useful to consider susceptibility as separate and distinct, as a biological characteristic, from resistance.

(2) Intraspecific natural resistance to infection is operationally and conceptually a function of the variational structure of the pathogen population.

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ACTIVE AND PASSIVE IMMUNITY OF THE INFANT

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In a complicated field such as the active and passive immunity of the infant one cannot readily be encyclopedic. The usual limitations of space are, in this case, enhanced by the long span of time covered by pertinent studies of the topic at hand. There are excellent sources of background information on the subject, such as the studies of Ratner, Jackson, and Gruehl,[1] the chapter on "Natural Antibodies" in Topley and Wilson's *Principles*,[2] and the excellent, up-to-date review sections in a recent monograph by Brambell, Hemmings, and Henderson.[3] In addition, several other papers included in this monograph deal with specific aspects of immunity in the newborn. I shall confine myself, therefore, largely to the citation of selected findings that illustrate or emphasize the turning points in our knowledge or our speculation in this field.

Awareness of the existence of immunity to infectious disease in the newborn no doubt goes back to antiquity. Recognition of this immunity as a scientifically observed phenomenon, however, appears to have arisen along with the development of experimental immunization in the 1870's. Bollinger[4] may perhaps deserve credit for making the first observations of this type, having reported in 1877 that lambs delivered of recently vaccinated ewes were immune to cowpox. This finding was soon confirmed by Burckhardt.[5] Chauveau[6] demonstrated soon afterward that immunity to anthrax could be established in lambs by vaccinating the ewes. Ehrlich similarly showed that maternal immunity to various toxins could be transmitted to newborn mice.[7, 8] There followed a series of observations on the transmission of antibodies to diphtheria in guinea pigs[9] and in man,[10, 11] and to tetanus in man,[11] even before Theobald Smith's famous report of this finding in guinea pigs.[12] Rosenau and Anderson then demonstrated that sensitivity to horse serum could be similarly transmitted in the guinea pig[13] simultaneously with passive immunity to diphtheria toxin.[14]

Colostrum and milk. In his original studies[7] Ehrlich, in beautifully designed experiments, presented evidence that the transmission of passive immunity from mother mice to their offspring was mediated in part through the placenta and in part via the mother's milk. Little attention seems to have been paid to the question raised by these experiments, until Famulener[15] showed that artificially induced hemolysins in goats were transmitted to their young through the ingestion of colostrum. The clinical importance of this mechanism was later strikingly shown by Smith and Little,[16] who found that about 75 per cent of newborn calves, if deprived of colostrum, would die of *Escherichia coli* septicemia, whereas even a single brief ingestion of colostrum resulted in survival. Little and Orcutt,[17] in a parallel study, found that *Brucella abortus* agglutinins were transmitted from cow to calf via colostrum. Howe[18] demonstrated that the ingestion of colostrum was rapidly followed by the appearance of euglobulin and pseudoglobulin in the calf's serum—a finding much later confirmed in calves and lambs by ultracentrifugal and electrophoretic studies.[19-22] Hansen[21] later found that the absorption of colostrum by the calf—as measured by electrophoretic analysis of the serum—could take place only during the first 24 hours after birth. When colostrum was withheld, the various serum protein fractions rose only slowly, approaching normal at about eight weeks *post natum.* It is of interest that the immune globulin derived from colostrum is electrophoretically distinct from normal serum euglobulin, and that this distinct "immune lactoglobulin" can be identified as such in the serum of calves fed colostrum.[22] Moreover, it has recently been shown that the protective action of bovine colostrum is antigenically specific, and will apparently give good protection only against those coliform types for which it carries the corresponding anti-K antibodies.[23] The over-all problem of immunity to "scours" in young calves continues to expand in interest and complexity; it has recently been broadly reviewed by Lovell.[24]

There have been many studies showing the importance of colostrum in the establishment of immunity in various ruminants, notably those of Mason *et al.*[25] in lambs, calves, bitches, and mares, and in similar studies by other investigators (*e.g.*, Barr, Glenny, and Howie[26]).

Lemétayer and his associates have examined many of the details of the phenomenon of colostrum-transmitted immunity. They have shown that antibodies transmitted via colostrum appear (in foals) within an hour after the first neonatal nursing;[27] that the antibody level in the colostrum (in mares) falls rapidly within less than 24 hours after the birth of the foal;[28] and that—in general harmony with the findings of Hansen[21] and of Mason *et al.*[25]—the ability of the foal to absorb detectable amounts of antibody by this route disappears relatively early.[29] Lemétayer *et al.* also demonstrated, in passing, that some antibody was transmitted from mare to foal by way of the placenta.[30] An interesting sidelight on these findings is the recent demonstration[31] that foals, like human infants, can be afflicted with a hemolytic state induced by maternal antibodies against fetal erythrocyte antigens. In this instance, the isoantibody is apparently transmitted via colostrum, as would be expected.*

A question has arisen as to whether the large quantities of antibody transferred by some animals by the mammary route are made available by direct secretion of serum antibodies, or by resynthesis of antibodies by the mammary glands. Askonas *et al.* using a radioactive tracer technique, have neatly demonstrated that, at least in rabbits and goats, the former explanation is correct.[32] Following the course of colostral antibodies further, it has been found (in calves and kids) that the antibodies enter the circulation via the intestinal lymph tracts and the thoracic duct.[33]

The reports by Lewis and Wells[34] and by Boyd,[35] in 1922, that ingestion of colostrum led to a rise in serum euglobulins in the human newborn infant resulted in considerable interest in the possible prophylactic importance of human colostrum. It has indeed been shown[36, 37] that human colostrum may contain antibodies. Their titer is low at best, however, and more frequently they are absent. (The astoundingly high titers of antityphoid antibodies in human colostrum reported by Schubert and Grünberg[38] appear to be an exception, but these findings require confirmation.)

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* It may be appropriate at this point to note that the subject of erythroblastosis—illustrating, as it does, a mechanism whereby the transuterine passage of antibodies induces disease in the fetus and the newborn infant—might deserve a major place in this review. The subject of erythroblastosis is such a large one, however, and has been so thoroughly dealt with elsewhere, that it will not be given further consideration here.

Römer noted many years ago that antibody levels in the mother and the newborn infant were generally comparable in guinea pigs, rabbits, and man, implying transplacental passage of antibody. On the other hand, antibodies did not appear to reach the cow, sheep, or horse fetus prior to birth.[39] The classical studies of Kuttner and Ratner[40] still stand as the best description of the problem. These investigators pointed out that goats, cows, and other ruminants, which transmit significant amounts of antibody through colostrum, possess complex placentas having multiple layers between maternal and fetal circulations; whereas rodents and primates, in which transplacental transmission of maternal antibodies appears to be the dominant mechanism, have only a single tissue layer between the two circulations. This concept has been confirmed by the extensive experiments summarized by Schneider and Szathmáry[41] in hogs, goats, horses, cows, sheep, dogs, rabbits, and guinea pigs.

Passive immunization of the newborn by ingestion of antibodies in milk has continued to receive intermittent attention since Ehrlich's original studies, having been shown to apply in immunity to a protozoal infection[42] and a viral infection.[43] Lemétayer *et al.* found that, in cattle immunized with tetanus toxoid, the antitoxin titers of their milk ran from about $\frac{1}{100}$ to $\frac{1}{6}$ the titers in the corresponding sera.[44] Witebsky *et al.*[45, 46] have demonstrated the presence of anti-Rh antibodies in human breast milk. The significance of Witebsky's findings is questioned, however, by Bowman,[47] who attempted unsuccessfully to induce measurable isoagglutinins in infants by feeding them high-titer anti-Rh serum in large quantities.

Placental transmission. As was clearly shown by Kuttner and Ratner,[40] passive immunity in the newborn human being is mediated almost entirely via the placenta. Ten Broeck and Bauer[48] demonstrated the transmission of tetanus antitoxin from mother to infant through the placenta a number of years ago. McKhann and his associates (*e.g.*, McKhann and Chu[49]) demonstrated the presence of diphtheria antitoxin and other antibodies in placental blood, later applying this principle to the control of measles by injecting the antibody-containing fraction of placental extracts. Numerous other antibodies have been shown to be transmitted from mother to human offspring under conditions that exclude any known mechanism except the uterine route. Most of such studies are based upon titrations of maternal versus umbilical-cord sera, thus ruling out the possibility that the results could have been influenced by ingestion of colostrum.

Quantitatively, however, the findings show considerable variation. For example, Sherman, Hampton, and Cooke[50] had found that allergic sensitizating antibodies or "reagins" were not transmitted, to any detectable degree, via the placenta, whereas allergic "blocking" antibodies were transmitted more or less to titer. Moreover, Wiener and Silverman found that conventional isoagglutinins were present in infant's serum, but only to a titer of about $\frac{1}{8}$ to $\frac{1}{16}$ that found in the mother.[51] In later studies,[52, 53] however, they showed that blocking-type antibodies were usually to be found in approximately equal titers on either side of the placental barrier. Nevertheless, the much more extensive observations of Zuelzer and Kaplan[54] indicate that the difference in placental transfer between the two types of antibody is by no means as clear-cut as suggested by Wiener's observations. Many studies have been done on the ratio of antitoxic, antibacterial, or antiviral antibodies in maternal and cord sera. Among the more recent reports of this nature, Florman, Schick, and Scalettar[55] found that the maternal/cord ratio of mumps antibodies was 1:1, whereas the ratio for *streptococcus* MG antibodies was greater than 1:1. A variety of similar findings, pertinent to numerous different types of antibody, has accumulated in the literature. Many of these findings, ranging from 1:1 ratios, on the one hand, to the passage of little or no antibody into the cord blood on the other hand, have been tabulated in a study by Vahlquist *et al.*,[56] who themselves found that the maternal/infant ratio for antistreptolysin and antistaphylolysin in mature infants was in the neighborhood of 1:1. The later findings of Adamson *et al.*[57] are in agreement as regards these antitoxins, and add the further interesting observation that, in the same infants, *E. coli* H antibodies pass only moderately, and O antibodies scarcely at all, from mother to infant.

On the other hand, Barr and her associates observed, several years ago, that cord-blood titers of diphtheria antitoxin were generally up to 50 per cent *higher* than the maternal titer.[58] This anomalous finding was at first thought to be due to some technical accident (*cf.* Wiener[59]). A similar observation, however, was recorded by Liebling and Schmitz[60] several years earlier. Moreover, Bryce and Burnet,[61] over 20 years ago, had observed this in studies on antistaphylolysins. Furthermore, several analogous observations in maternal and newborn sera have also been recorded for antistreptolysins[62-64] and for pertussis antibodies.[65, 66] More recently, Murray *et al.*,[67] working with staphylococcal antitoxin, found that cord-blood titers averaged 63 per cent higher than those of maternal blood. Kempe and Benenson[68] found, similarly, that a high proportion of the cord-blood titers of vaccinal antihemagglutinins that they measured were higher than the corresponding maternal levels. Indeed, Doerr,[69] in a masterly review of the problem of placental permeability, called attention some years ago to the possibility that this organ may be capable of concentrating antibodies.*

Some of the findings just cited resulted from attempts to induce neonatal immunity by immunizing the pregnant mother, so as to insure the transmission of a protective titer of antibody to the infant. This technique has been directed primarily toward prevention of whooping cough,[65, 66, 70-73] diphtheria,[60, 73] and influenza.[73] The results obtained by this process have been as variable as the previously cited observations would suggest. Kendrick *et al.*[71] did observe, however, that the higher the maternal titers induced, the nearer the neonatal titers approached those of the corresponding mothers.

Mechanism of transmission. The great variation thus seen in the transmission of maternal antibodies to the infant—ranging from apparently none in some cases to paradoxically high values at the other end of the spectrum—has led to considerable speculation on the mechanism of this transfer. It would appear from the findings of Vahlquist *et al.*[56] on antibody titers in premature infants that the transmission of antibodies to the infant was quite variable until about the 30th week of gestation, after which the 1:1 titer ratio that they found appeared to prevail. In a more extended study, Osborn, Dancis, and Rosenberg[75] plotted maternal-infant diphtheria antitoxin titers in 56 fetuses or premature infants ranging from 50 to 2600 g. in weight. At fetal weight of 400 g. the mother had, on the average, as judged from the author's regression line, about 0.7 log more antitoxin than the infant; whereas at 2600 g. the maternal level averaged about 0.2 log less than that of the infant. Their findings may perhaps be related, at least in part, to the observations of Moore and his colleagues[76] that the gamma globulin as well as the albumin levels in fetal blood start at low levels and rise steadily toward term. It may be assumed that the placental transfer of antibody globulin from mother to infant takes place at a rate faster than the destruction of antibody in the infant circulation, so that the levels of those antibodies that can be transferred will steadily rise toward term. The great discrepancies in degree of transmission, however, require a more detailed explanation. Wiener[53, 59] has suggested that "bivalent" type or "complete" antibodies are held back at the placental barrier, whereas "incomplete" or "univalent" antibodies, being perhaps smaller, can cross the barrier. This thesis, insofar as it might apply to isohemagglutinins, derives some support from the recent finding that the "complete" type of Rh antibody appears to have a higher sedimentation constant than does the "incomplete" type of antibody.[77] The problem, however, is not as simple as this. The various quantitative findings cited above do not fit readily into any simple hypothesis, and observations directly contradicting Wiener's theory have been made by Hartley,[78] who found that unaltered guinea pig antibody was much more readily transmitted to the offspring than was antibody that had been pepsin-digested and was therefore presumably smaller.

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* These findings of unexplainably high cord-blood antibody titers may be related to the finding made by some investigators[69a, 76] that gamma-globulin levels in cord blood are considerably higher than in the mother.

Some years ago McKhann[49] pointed out that the then available data suggested that the antibodies that passed the placental barrier most readily were pseudoglobulin in nature, whereas those that passed with difficulty, if at all, were in general euglobulins. Subsequently, Witebsky *et al.* observed that "complete" Rh antibodies had the solubility characteristics of "euglobulins," the incomplete antibodies behaving as pseudoglobulins.[79] Thus McKhann's suggestion has at least some experimental support, and it deserves re-examination in the light of the present knowledge of the nature and interactions of antibody molecules. It would still leave unexplained, however, the repeated finding that the infant's antibody level may be higher, as much as 50 per cent higher, than that of the mother.

Aside from Doerr's supposition cited above,[69] few contributions toward the understanding of this problem have been offered. The studies of Brambell and his associates, however, on the mechanism of antibody transmission from mother to young in the rabbit offer a completely new possibility. They have found[3, 80] that agglutinins can pass from the maternal circulation into the uterine lumen, whence they pass through the entodermal membrane of the yolk sac into the vitelline circulation. The role of absorption through the stomach in the rabbit has apparently been largely excluded by the studies of these investigators, who find definite evidence against the concept of transplacental transmission of antibodies in this animal. Regardless of the detailed differences that may prevail between species in these respects, the findings of Brambell *et al.* open up the possibility that routes of transmission other than the placental route may be of importance in rodents, and possibly also in primates.

The immunity status of the newborn is thus a complex of many factors, among which the availability of maternal antibodies, whether transmitted by colostrum, milk, the placental circulation, the yolk sac, or the amniotic fluid, is of fundamental importance in establishing the ability of the infant to survive the first few weeks of life. Aside from antibodies present at birth, few other factors have been studied. Traub[81] found a wide scatter of complement levels in newborn infants, as compared to the corresponding maternal levels. The general level in the infants, however, was no more than 50 to 60 per cent of that in the mothers. Dancis and Kunz,[82] studying the bacteriostatic and complement activity levels in premature and mature newborn infants, found relatively high bacteriostatic activity, falling off to a low at between one and four months, followed by a subsequent rise. It is not clear whether they were testing complement or a complex of factors, since the heat lability of their factor and the factors that could replace it are not entirely consistent with either complement or antibody. It would be interesting to know what the levels of properdin activity are in infants.

Dancis and Kunz noted that the fall and subsequent rise, with the passage of time, of bacteriostatic activity observed in their subjects, paralleled the fall and rise observed in gamma-globulin levels in infants. The course of the serum gamma-globulin level in young infants was clearly defined by Moore *et al.*[76] It has been quantitated by Wiener,[83] and (for premature infants) by Norton, Kunz, and Pratt.[84] The initial level at birth is comparable to that of the mother[83] or higher.[69a, 76] In the first three months it may drop to about one third of this level, after which it slowly rises again. A comparable fall has been found by Barr *et al.*[58] and by Wiener[59] to occur in antibody levels in the young infant. Their studies showed that the degradation of maternally transferred antibody followed the course of a first-order reaction, with a half-life of about 30 days—a value that, by the way, is considerably longer than that now generally accepted for adult human beings.[85]

Active immunization. It is perhaps fortunate that the infant's capacity to break down homologous antibody proteins derived from the mother is slow, as compared to that of the adult, for it is obvious that the passive immunity provided by maternal anitbodies plays a major role in protecting the infant until it can develop its own mechanism for antibody formation and thus respond successfully to the antigenic stimuli and infectious insults that assail it. This response, too, appears to be somewhat less actively developed than it is in the adult. Various past studies, notably those of Freund[86] and Baumgartner[87, 88] have indicated that the capacity to form antibodies is impaired in the young rabbit. The evidence, however, for such impairment is by no means consistent. The widespread impression that human infants responded poorly to diphtheria antigens or, for example, to smallpox vaccination[89] has been re-examined in the light of the interfering effect of persisting maternal antibodies in the infant.

Cooke[90] administered combined diphtheria and tetanus toxoids to infants from 1 to 14 months of age. The response to tetanus toxoid showed no significant relation to age, the infants under 3 months doing as well as the older groups. With diphtheria toxoid this was not the case for, when the younger infants were sorted in accordance with the presence or absence of maternally transmitted diphtheria antitoxin, it was found that those without inherited antitoxin responded well to the diphtheria antigen, whereas those with 0.03 unit of antitoxin or more responded relatively poorly. Essentially similar findings have been made by Barr and her associates,[91, 92] and by Greenberg and Fleming.[93] The latter investigators found, moreover, that the antibody response in infants with pre-existing antitoxin was at least one half that of the infants without such an interfering factor—a value that greatly modifies the widespread but unsupported impression that those with pre-existing antibody do not respond. In fact, they found that the presence of inherited antibodies did not prevent an *adequate* response to a good antigen, but simply delayed it[94]—a finding like that reported earlier by Vahlquist *et al.*[95] In this connection, Vahlquist and Nordbring[96] and Dancis, Osborn, and Kunz[97] have measured the antibody response of premature infants and have found that it did not materially differ from that of full-term infants.

Numerous other studies on immunization of young infants have contributed valuable information and have partly clarified a field previously clouded with uncertainty. Over a decade ago Keller, Peterson, and Densen[98] immunized young infants against whooping cough. They obtained immune responses only slightly below those of older infants. The recent development of combined immunization has intensified the interest in neonatal immunization. Di Sant' Agnese commenced immunization with adsorbed diphtheria-tetanus-pertussis antigen one week after birth and followed the response to three doses,[99] the effect of a booster,[99, 100] and the comparison of the response with that in infants 6 to 12 months old.[101] He found that the responses to diphtheria and pertussis antigens were generally better in older infants, the booster response to the pertussis component was better in the older infants, and that (at least for diphtheria) the poorer response in younger infants could be ascribed to the interfering effect of maternal antibodies, as noted by others (see above). The responses of the young infants, however, were in general so good that he concluded that neonatal immunization was of great practical value. Fundamentally similar findings have been made by Petersen *et al.*,[102] working with infants under three months of age, and Osborn *et al.*[103] in infants of various ages, the youngest of which were two weeks old. As regards another long-noted difficulty in neonatal immunization, vaccination against smallpox, Kempe and Benenson[68] find that this, too, is probably due to passive immunization of maternal origin, a finding not inconsistent with the frequent complete success of smallpox vaccination of newborn infants in actual pediatric practice.

Conclusions. Various other studies that bear on this question could be cited if space permitted. All the studies recorded above, however, seem to add up to a few fairly definite conclusions, namely:

(1) Passive—and hence transient—immunity is transferred from mother to young in various animal species by various mechanisms: mainly via colostrum

in ruminants, and probably mainly via the placenta in rodents and primates. The latter conclusion is subject to possible revision in the light of such findings as those of Brambell *et al.*[3, 80]

(2) The degree of transfer of passive immunity varies tremendously from one antibody to another. This variation is in all probability related to the molecular characteristics of the various kinds of antibodies present in the maternal circulation.

(3) Consequently, the newborn infant will acquire protection of varying degree against certain infections in which serological factors play a significant role in resistance. On the other hand, there will be some infectious processes, such as whooping cough, against which maternally transfered antibodies, even though frequently present, are generally insufficient in titer to furnish effective protection. For certain of the infectious hazards of the newborn, added protection during the neonatal period may be accomplished by immunizing the mother before or during pregnancy.

(4) The antibodies available to the newborn infant by one or another of the mechanisms above mentioned are catabolized and disappear during early infancy, leaving the infant more or less wholly susceptible to the infectious processes native to its age and species. Subsequent survival must depend largely upon the acquisition of active immunity, either by surmounting natural infections or by artificial immunization. Active immunization is to some extent interfered with by the presence of passive immunity, but this interference can generally be overcome by the administration of sufficient antigen.

(5) Over and above the known factors in neonatal immunity, summarized above, there is a host of unknown factors. Some of these are: the generally assumed hypoplasia of the reticuloendothelial system in very young infants (for which there appears to be little histological support); the obvious lag in the developmental production of gamma globulins in young infants; the varying concentrations and activities of passively or actively acquired complement components, properdin, *etc.*; the vague and intangible but nevertheless recognizable phenomenon sometimes described as "tissue immunity of the young"[104] or the natural resistance of the young, which has recently been re-examined in a lecture by Lovell.[105] Then there is the even more intangible but not yet entirely discarded concept of "natural antibodies" acquired as part of the maturation process. Some of these factors may be significant, other may prove to be merely figments of our fertile imaginations, and still others, of which we yet know nothing, will arise, perhaps to supersede much of our present system of thinking in this field. At all events, the field has not yet been plowed deeply, despite 75 years of activity. It remains for us and our students to pursue more thoroughly the basic facts that characterize this special branch of immunology. Like most of immunology, the surface may sometimes appear deceptively simple, but the basic understanding of the phenomena is yet to be gained. More power to those who recognize and exploit this opportunity!

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SALMONELLA AND SALMONELLOSIS

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The study of *Salmonella* and salmonellosis has a long and varied history. During the nineteenth century the relationships of *Salmonella* to animal diseases, to enteric fever of man, and to mass outbreaks of food poisoning were recognized. At this early period it became evident that certain types were associated rather constantly with enteric fever of man while other types were associated closely with specific diseases of certain animals. The isolation of closely related types from a variety of morbid conditions in man and from many species of animals, however, produced a degree of confusion seldom equalled in the annals of bacteriology. Shortly after the turn of the century, workers in Britain, particularly Boycott,[1] and Bainbridge and O'Brien,[2] applied agglutinin absorption tests to the differentiation of types, while the investigators at Kiel, Germany, led by Bernhard Fischer, Reiner Müller, and L. Bitter, applied cultural and biochemical tests in the study of the bacteria. The latter work was summarized by Pesch,[3] who pointed out the opposition aroused by Uhlenhuth[4] and others. Gradually there emerged an acceptance of the fact that *S. paratyphi* B was a human pathogen passed from man to man and that, in man, it produced enteric fever, while *S. typhimurium*, the Breslau bacillus, produced a variety of diseases in animals, and in man was responsible for mass outbreaks of food infection characterized by acute gastroenteritis. This dictum was widely known as the "Kiel doctrine."

It was not until Bruce White[5, 6] and Kauffmann[7, 8, 9] established the present method of classification that the antigenic characteristics of the *Salmonella* group and its great diversity of serotypes and biotypes became known. These advances made possible the exact characterization of *Salmonella* types and the numerous subsequent studies on the ecology and epidemiology of the group. It soon became apparent that within the group there existed types that were rather strictly host-adapted either to man or to certain species of the lower animals and that, with notable exceptions, the occurrence of these forms was restricted to their specific hosts. Further, there existed a much larger number of types, now approaching 400 in number, that exhibited no host specificity, and that, upon occasion, might produce disease in any warm-blooded animal. It is with the latter group of cultures that the present paper largely is concerned.

Improved methods of isolation and characterization stimulated the study of *Salmonella* infections. It soon became evident that the organisms were widely distributed throughout the animal kingdom and that the same serotypes occurred both in infections of man and of animals.[10, 11] It further was noted that in the great majority of instances the bacteria were found in sporadic infections and not in widespread outbreaks involving large numbers of persons. The sporadic character of the infections was emphasized by Smith,[12] Hormaeche *et al.*,[13] Neter[14] and, more recently, by Eisenberg and his associates.[15] The sporadic nature of the cases, the frequency with which children were attacked, and the diversity of symptoms in young children prompted Hormaeche to propound the "Montevideo doctrine," as opposed to the "Kiel doctrine," which largely took into account only well-defined outbreaks of food infection.

In a consideration of the epidemiology of the infections due to nonhost-adapted types of *Salmonella*, two main sources of infection must be considered: reservoirs of infection among lower animals and human carriers of the bacteria. Our own experience in the examination of more than 30,000 *Salmonella* cultures has impressed upon us the frequency with which these organisms occur in animals and in asymptomatic persons, and the diversity of types found in both. The impressions regarding lower animals are confirmed amply by the voluminous literature dealing with salmonellosis of domestic and wild mammals and fowls. Although other animals harbor, and occasionally transmit the infections, principal emphasis must be placed on those animals used widely as food.

Ruminants. In most parts of the world *S. dublin*, a close relative of *S. enteritidis*, occurs commonly in cattle. Other types, particularly *S. typhimurium*, also are found in these animals. The prevalence of *Salmonella* infections and of carriers of *Salmonella* in cattle was emphasized by Bartel,[16] Henning,[17] Field,[18] and many others. It is known that many healthy cattle are either temporary or permanent carriers of the bacteria, that large numbers of the bacteria are excreted in the feces and that, occasionally, mastitis occurs in infected cows. The role of the infected cow in milk-borne epidemics of salmonellosis was reviewed by Savage,[19] by Henning,[20] and by McCall.[21] While the majority of such outbreaks have been caused by *S. dublin*, Norton and Armstrong[22] point out that *S. typhimurium* also may be involved. Objection may be raised that the outbreaks described were caused by the consumption of raw milk and that proper pasteurization would have prevented them. It must be remembered that throughout the world a large proportion of milk consumed still is not pasteurized. Further, the mere passage of milk through a pasteurizing machine does not automatically ensure its safety. One of the most extensive outbreaks of *S. typhimurium* infection that has occurred in this country in recent years was that described by Tucker *et al.*,[23] in which the vehicle was cheese prepared from "pasteurized" milk. In passing, it may be mentioned that cows, as well as chickens, sheep, and swine, sometimes become infected with the "human" type of *S. paratyphi* B[10, 24] and occasionally are responsible for milk-borne outbreaks of paratyphoid B fever.[25] The role of beef in the epidemiology of food infections has been recognized since the days of Gärtner[26] and of Basenau.[27] The part played by beef in the production of salmonellosis recently was reviewed by Clarenburg.[28] The recent widespread epidemics in Sweden also should be mentioned. As regards other ruminants, it is known that sheep frequently are infected with *S. typhimurium* and a variety of other *Salmonella* types.[10, 29, 30]

Swine. Swine long have been notorious as carriers of *Salmonella* but it was not until Hormaeche and Salsamendi[31, 32] systematically examined the lymph glands of apparently normal hogs that the number and variety of the organisms in these animals were fully realized. This work was amply confirmed by many workers, including Rubin[33] and Galton *et al.*[34] The latter workers noted that isolations from swine in abattoirs were much more frequent than from animals on the farm, and mentioned the importance of the sales yard, the holding lot, and the abattoir in the spread of the organisms. Numerous instances of the transmission of salmonellosis by pork products are to be found in the literature. One of the most unequivocal of these is the report of Hauser *et al.*[35] on infection by *S. berta* following consumption of contaminated smoked sausage. That such infections do not occur more frequently is surprising since Galton, Lowery, and Hardy[36] isolated *Salmonella* from 23 per cent of the samples of fresh sausage and 12.5 per cent of the samples of smoked sausage examined.

Fowls. Considering the extremely large population of domestic fowl, birds probably constitute the largest single reservoir of *Salmonella* among animals.[10, 37, 38] It is evident from the results of Gibbons and Moore,[39] of Solowey and her associates,[40, 41] and of the investigators of the Medical Research Council, London, England,[42] that the bacteria frequently gain entrance to eggs and egg products. The role of duck eggs in the production of human salmonellosis has been reviewed by Clarenburg[43] and by Lerche.[44] Duck eggs have been one of the important sources of *Salmonella* infection in Holland and in the West German Federal Republic for many years. Recently, episodes of this sort were reported in England.[45] Chicken eggs also have been incriminated, by Watt[46] and others, in the transmission of *Salmonella* to man. *Salmonella* may gain entrance to eggs either as a result of ovarian infection or by penetration of the egg shell. Their frequent occurrence in processed eggs was attributed largely to egg-shell contamination in the investigation by Solowey *et al.*,[47] who emphasized the desirability of using only clean eggs in processing plants. It has been shown by Solowey and Calesnick[48] and by the British workers[42] that *Salmonella* in reconstituted eggs may survive cooking procedures. It is well known that many egg products used in bakeries are subjected to only slight heating. It becomes evident, therefore, that ample opportunities arise for the transmission of *Salmonella* through the medium of eggs and egg products.

Eggs are not solely responsible for the transmission of avian salmonellosis to man. At least two other modes of spread are involved. Transmission through the handling of diseased fowl has been reported repeatedly.[10, 49, 50] Further, carcasses and edible viscera of birds entering commercial channels have yielded a variety of *Salmonella* types.[51, 52] These contaminated carcasses often give rise to infection in man. Such occurrences were summarized by Browne[53] and by Hinshaw and McNeil.[54] Not only may poultry and poultry products themselves serve as sources of salmonellosis in man, but fowls may infect other foods as well. Bidwell and Kelly[55] recorded the contamination of oysters with *S. typhimurium* by drainage from contaminated duck farms.

Other animals. Rodents were long believed to carry only *S. typhimurium* and *S. enteritidis*. While this is still true of laboratory rodents, wild rats now are known to harbor a variety of serotypes.[56, 57] In the experience of Ludlam,[58] rodents are prone to carry those types of *Salmonella* that are present in their environment. No longer is it reasonable to suspect rodents, particularly, in *S. enteritidis* infections of man, since rats and mice are just as likely to transmit any of the serotypes occurring commonly in man and other animals.

Dogs have been incriminated a number of times in the transmission of *Salmonella* to man.[59, 60, 61] Galton, Scatterday, and Hardy[62] found *Salmonella* in 27.6 per cent of all dogs examined and in 15 per cent of apparently normal household dogs. This incidence is probably attributable in large part to the presence of the organisms in the food of the animals, since Galton, Harless, and Hardy[63] found *Salmonella* in 26.5 per cent of the samples of commerical dog meal examined, and Griffin[64] noted *Salmonella* in a lesser number. In this connection, it is of interest to note that Griffin also isolated *Salmonella* from feed supplied to mouse breeders and from feed designed for guinea pigs. The difficulties of maintaining rodent colonies free of the infections quickly become apparent.

Insects of various sorts, particularly flies, have long been suspected of being agents of transmission of *Salmonella*. The organisms have been found in flies on a number of occasions.[13, 65, 66] From the results obtained by Watt and Lindsay[67] in fly-control studies it would seem that flies are of less importance in the transmission of *Salmonella* than of *Shigella*. Cold-blooded animals such as snakes[66] and tortoises[68] have been found to harbor *Salmonella*. It has been suspected that the former transmitted the organisms to fowls. Finally, to illustrate the ubiquity of *Salmonella*, one might cite the observation of Nakaya,[69] who described an extensive outbreak of salmonellosis among villagers who captured an infected whale and consumed the meat, which contained *S. enteritidis*.

Man. From the foregoing summary it is evident that numerous *Salmonella* types are widely distributed throughout the animal kingdom. From the aforementioned reports of *Salmonella* in eggs, egg powder, and sausage, and from the publications of Cherry *et al.*[70] and of Felsenfeld *et al.*,[71] it is evident that *Salmonella* find their way into commercial meats, particularly pork and poultry products. It must not be assumed, however, that animal reservoirs of infection are solely responsible for endemic salmonellosis of man. First, not all contamination of meat products can be attributed to the presence of the bacteria in animals. The work of Floyd *et al.*,[72] in which *Shigella*, *Salmonella typhi*, and *S. paratyphi* A were found repeatedly in meats, forcibly illustrates contamination of human origin of meat products in an area of poor sanitation and high endemicity. Further, Clarenburg[28] emphasizes the necessity of differentiating between intravital and post-mortem contamination, and points out that processed meat is much more often responsible for food infection than fresh meat.

For many years the frequency with which the nonhost-adapted *Salmonella* occurred in asymptomatic persons was not realized, and it was thought that the organisms disappeared rapidly from the intestine of convalescent cases. It now is known that these types are present in many persons without apparent enteric infection,[10, 11, 73] and that the organisms may persist in such persons and in convalescents for long periods.[74, 75] Many transient asymptomatic carriers also occur in the population, as demonstrated by Galton and Hardy.[76] Carriers among young children have been found frequently by Watt and Lindsay[67] and by Mackerras and Pask.[77] Galton and Quan[78] noted the prevalence of *Salmonella* among food handlers. It seems probable that the frequent occurrence of *Salmonella* in market meats contributes to the carrier state in food handlers. The carrier state probably could be called an occupational hazard of food handlers. The importance of the asymptomatic carrier in the transmission of salmonellosis was emphasized by Felsenfeld and Young,[79] who reported that 26 of 56 outbreaks, the sources of which were established, were caused by human carriers. Outbreaks due to *S. typhi*, *S. paratyphi* A, and *S. paratyphi* B are excluded from these figures. Convalescent carriers often have been incriminated in the transmission of the infections. One such outbreak was described by Rubenstein and MacCready,[80] who emphasized the large number of symptomless excretors among persons who ate the contaminated food. The constant presence of *Salmonella* in sewage and in irrigation waters[81, 82] also attests the frequent occurrence of the organisms in man.

Thus it is evident that both animal reservoirs of infection and human cases, convalescents and carriers, are concerned in the epidemiology of salmonellosis, and each should be given due consideration in any program of prevention. It is obvious also that salmonellosis in infants is a reflection of the occurrence of the bacteria in man, animals, and the environment. Inasmuch as the young of all species are particularly susceptible to salmonellosis, infections in infants present a special problem.

The presence of the organisms in nurseries has been especially troublesome. Introduction of infections into nurseries by babies born of mothers who were preclinical cases, convalescents, or carriers has been noted by Watt and Carlton,[83] Abramson,[84] Seligmann,[85] Neter,[86] and by Epstein *et al.*[87] Unpublished episodes of this sort repeatedly have been brought to our attention. It seems highly probable that this method of transmission is not infrequent. Obviously any mother who exhibits the slightest symptoms of enteric infection or who has recently recovered from such an infection, as well as the babies of such mothers, should be subjected to strict isolation until such time as it is determined that they are not affected with a transmissible disease. Carriers among the food handlers of hospital staffs have been noted by Neva, Nelson, and Finland,[88] and these constitute an additional hazard in the nursery. If food handlers in general are to be subjected to systematic fecal examinations, surely it is not too much to ask that food handlers in hospitals be subjected to special scrutiny, since the population endangered, being composed in large part of the very young and the infirm, is especially susceptible to enteric infections. This requirement should apply also to other staff members in contact with infants. Staff members and food handlers affected with any symptoms of enteric infection should be relieved of duty until it is determined whether or not they have a communicable disease. Similar recommendations were made by Felsen, Weil, and Wolarsky,[89] who further advocated fecal examination of all parturients.

Infants transferred into obstetrical nurseries from other hospitals have been found by Watt[90] to introduce salmonellosis. Likewise, Wright *et al.*[91] and Rogers and Koegler[92] noted the introduction of enteric infections into pediatric wards by newly admitted infants. It seems advisable to isolate all new admissions to such wards until it has been determined that they are not excreting known pathogenic enteric bacteria. Within a given metropolitan area there should exist an interhospital intelligence service, to report not only on enteric infections but on other communicable diseases as well. Such a service could be of great value in preventing the introduction of infections into nurseries.

Questionable practices occasionally have given rise to epidemics. In one instance two babies affected with diarrhea and later found to be infected with *Salmonella* were promptly removed from an obstetrical nursery and the nursery closed to new admissions. No further cases developed. The infected infants, however, were placed in isolation in a room adjacent to rooms housing premature babies. Seven cases developed among the latter, and three fatalities were recorded. In a second hospital, located in the arid Southwest, a series of unrelated cases of obscure origin occurred in succession. Investigation by the State Department of Health revealed that the lawn of the hospital had been fertilized with dried activated sludge, and from it were isolated the same *Salmonella* types that were found in the patients.[93] In either of these instances, more thoughtful management would have prevented infections.

Accidental contamination of objects in delivery rooms and nurseries occasionally gives rise to outbreaks. Rubenstein and Fowler[94] noted the transmission of salmonellosis by infected resuscitators in the delivery rooms of two hospitals. Epstein *et al.*[87] reported the transmission of salmonellosis through the agency of a water bath used to warm formula bottles. The water bath was found to have been infected by an electrician who repaired the thermostat and who was an asymptomatic carrier. Such incidents serve to illustrate the manifold paths by which enteric pathogens may gain entrance to nurseries, and emphasize the need for frequent sterilization of all equipment. It immediately becomes apparent that in the investigation of enteric infections in nurseries every person and object with which the infants come into direct or indirect contact must be regarded with suspicion.

Recent observations on the spread of *Salmonella* infections in infants through such products as contaminated dried egg yolk,[95] soya milk, and dried yeast indicate that bacteriological control has not kept pace with the rapid development of food technology. Application of adequate safeguards to newly developed food products is a joint responsibility of the manufacturer and of governmental agencies.

Both Grant[96] and Hinshaw and McNeil[54] have published excellent reviews dealing with the epidemiology and prevention of salmonellosis. They are agreed that effective control can result only through cooperation of governmental agencies dealing with health, agriculture, and food. The responsibilities of the practicing veterinarian and the physician and the necessity of reporting both animal and human infections are pointed out. The examination and control of food handlers are emphasized. Some progress has been made in the elimination of the infections in cattle[18, 20] and in fowls,[97, 98, 99] but it must be said that these efforts at control have been dictated more by economic considerations than by regard for public health. It would seem reasonable to assume, however, that there will be a gradual improvement in the situation among domestic animals through improved animal husbandry. Improvement in the control of animal food products and other foods will probably also take place.

Such changes should be reflected in a lowered carrier rate in man and a gradual decrease in human *Salmonella* infections.

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AN EPIDEMIC OF *SALMONELLA PANAMA* INFECTIONS IN INFANTS

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The first isolation of *Salmonella panama* was by E. O. Jordan,[1] in 1934, in the course of an investigation of a food-borne infection among soldiers stationed in Panama. Subsequently, F. Kauffmann[2] identified the organism as a not previously described *Salmonella* and named it *S. panama.*

The first reference to *S. panama* as a cause of severe diarrhea, particularly of the very young, was by Schiff,[3] in 1938, in his description of this organism as a cause of diarrhea among infants in New York City.

In 1940, Katsampes and Bradford[4] reported the first case of *Salmonella* meningitis attributable to *S. panama* in a month-old infant.

The relatively infrequent mention of *S. panama* in medical literature as compared to other types, such as *S. typhimurium* and *S. paratyphi* B, prompts this report, which deals with the recovery of *S. panama* from 138 individuals during an investigation of an outbreak of diarrhea involving the nursery for the newborn in a hospital in a moderate-sized city in Michigan during 1943 and 1944.

The occurrence of three deaths from meningitis among newborn infants in the space of a week in a Michigan hospital during March, 1944, prompted the hospital administrator to request assistance from the Michigan Department of Health.

A review of the case reports and careful questioning of the obstetrical staff indicated that the nursery in question had been plagued with recurrent diarrhea for several months prior to the three deaths in question. Fortunately, the hospital had a competent pathologist on its staff, and autopsies prior to embalming had been secured on the three cases that terminated fatally on March 3, March 8, and March 9 respectively. Specimens of blood, spinal fluid, meninges, lung tissue, and feces had been obtained from each of the three autopsies and *S. panama* was recovered from all specimens by the *Salmonella* typing station of the Michigan Department of Health. All identifications were later verified by Edwards.[5]

A reappraisal of the hospital records for several months prior to this time indicated that between July 1943 and March 1944 at least four other infants had died in the same hospital with clinical and autopsy pictures that were compatible with the three deaths proved to be due to *S. panama* meningitis. The laboratory data on these four deaths indicated that a gram-negative bacillus had been isolated from each case, both from spinal fluid and from blood, but the organism had been considered, unfortunately, a contaminant; namely, *Escherichia coli.*

The hospital records also revealed that two other cases of *Salmonella* meningitis, that had been diagnosed in another hospital in the same city late in February 1944, had been born in the hospital under investigation on January 24 and January 28, 1944, respectively. Similarly, an investigation of the death of an infant from *S. panama* meningitis reported from a hospital in another city revealed that the deceased infant had been born June 4, 1943, in the hospital under investigation. Before the investigation was complete, 18 deaths were shown to be due to *S. panama* meningitis. All were infants under seven months of age except one white female who was 29 years of age and who died 18 days after undergoing elective surgery in the hospital in which the *S. panama* outbreak was occurring in the nursery. Thirteen of the group were less than 21 days of age.

The significance of these findings and the control measures instituted will be discussed later in this paper.

Clinical Data

The clinical picture common to this series of 18 was remarkably consistent: diarrhea, with greenish to greenish-yellow stools; vomiting; temperature in the range of 100° to 105° F.; convulsions; twitching of the muscles of the extremities; opisthotonos and/or generalized rigidity. All were listless or stuporous.

Four of the cases exhibited a rash similar to that observed in typhoid fever. Unfortunately, no attempt was made to recover *S. panama* organisms from the petechialike rash, but it is reasonable to assume that it was secondary to *S. panama* bacteremia.

Case 9 (R.W.) is representative of this series. R.W., a female child, was born February 20, 1944. She was released on the fourth day post partum, apparently normal, but was returned to the pediatric section of the hospital on the 13th day. The clinical picture was diarrhea and constipation alternately, listlessness, projectile vomiting, occasional convulsions, and a temperature that ranged from 103° and 105° F. Hiccups were also pronounced. The spinal fluid was under normal pressure, but cloudy, and contained 900 cells per cu. mm. *S. panama* was recovered from the spinal fluid. Death occurred March 8, 1944, the 18th day after birth.

Autopsy Findings

The anterior fontanel was bulging. On opening the cranial cavity and removing the dura mater, the cortex was not visible through the thick greenish-gray fibrino-purulent exudate that filled the subarachnoid space. The outer surface of the arachnoid membrane retained its glistening appearance. At the base of the brain, the exudate was so thick that it obscured the superficial brain structures. When the brain was sectioned, all sulci were filled with exudate, and the gyri of the cortex were swollen. The thoracic viscera were not remarkable. The intestinal tract was empty. The walls of the jejunum and the ileum had a milky appearance, with the Peyer's patches projecting prominently above the mucosa. The mesenteric lymph nodes were markedly hyperplastic. Specimens of blood, spinal fluid, lungs, liver, and contents of the colon were all positive for *S. panama*.

Control Measures

The fact that *S. panama* had already been identified in three cases of meningitis from babies who had been born in the hospital under investigation pointed strongly to the fact that we were dealing with an outbreak of *S. panama* meningitis of the newborn, associated with an outbreak of diarrhea due to the same organism. Working on this assumption until laboratory data could be obtained on the current cases, the same control measures were instituted as one would use for *S. typhi*. The whole obstetrical unit was placed under isolation, and stool specimens were ordered on all hospital personnel; namely, physicians, registered nurses, student nurses, nurses' aides, kitchen and diet kitchen employees, other employees such as floor maids, and all new admissions to the hospital.

Altogether, 24 individuals were found to have stools positive for *S. panama*. Of the 24 cases, 15 had close association with the obstetrical and nursery unit: 1 graduate nurse; 1 nurse's aide; 1 floor maid; 2 early cases of meningitis still in the nursery; 8 apparently well newborn babies, also still in the nursery; and 2 mothers of 2 of these apparently well babies. The other 9 positive individuals were: 1 adult male kitchen employee, 4 babies on the pediatrics service, and 4 babies who were on the outpatient service. The *S. panama*-positive individuals working in the hospital were relieved of their duties immediately and new personnel assigned only after they were proved to be negative for *S. panama*.

A new admittance and obstetrical unit was established in another wing of the hospital, staffed with new, highly trained personnel from the Diarrhea and Enteritis Study Group of the Bureau of Laboratories of the Michigan Department of Health, a cooperative program of the Michigan Department of Health and the W. K. Kellogg Foundation, Battle Creek, Michigan, for the study of diarrhea of the newborn. It is worthy of comment that this new unit remained uncontaminated throughout the long and trying experience of ridding the hospital and the community of *S. panama*. Some of the safeguards that contributed were: high-level supervision, bacteriologic screening of staff and new admissions, limitation of visitors, and terminal autoclaving of already capped and hooded formula bottles.

Epidemiologic Investigation

The discovery of 24 apparently well persons within the hospital, all positive for *S. panama*, suggested that the organism had been present in the nursery for a considerable length of time and suggested that the occurrence of fulminating *S. panama* meningitis was probably due to overwhelming infective doses. In an attempt to determine how long *S. panama* had been present in the nursery, the fatality from *S. panama* meningitis reported from the hospital in another city was used as a point of reference and an attempt was made to obtain stool specimens on all children who had been born in the hospital in question as far back as June 1, 1943. Letters were sent to the parents of these children, asking them to send in stool specimens from these children for laboratory examination. As could be expected, a varying number of specimens was received and TABLE 1 shows the number of births by months, the number of specimens received, and the percent of those received that contained *S. panama*.

Ten specimens were received from the 78 June births, none of which was positive. Fifteen specimens were received from the 88 July births, 2 of which were positive, or 13 per cent. Thirteen specimens were received from the 96

TABLE 1

FECAL SPECIMENS RECEIVED AND PER CENT POSITIVE FOR *S. PANAMA* BY MONTH OF BIRTH

	Births	Specimens	Positive	Positive %
1943				
June	78	10	0	0
July	88	15	2	13
August	96	13	3	23
September	111	15	4	27
October	92	12	5	42
November	75	7	3	43
December	91	15	6	40
1944				
January	93	14	8	57

August births, 3 of which were positive, or 23 per cent. Fifteen specimens were received from the 111 September births, of which 4 were positive, or 27 per cent. Twelve specimens were received from the 92 October births, of which 5 were positive, or 42 per cent. Seven specimens were received from the 75 November births, of which 3 were positive, or 43 per cent. Fifteen specimens were received from the 91 December births, of which 6 were positive, or 40 per cent. Fourteen specimens were received from the 93 January births, of which 8 were positive, or 57 per cent.

It became obvious that *S. panama* had been present in this hospital as far back as June 1943. From March 10, 1944, when the investigation was initiated, to April 15, 1944, *S. panama* was recovered from the stools of 20 infants and 1 adult female hospitalized for elective surgery. Of these 21, 7 of the infants and the adult female died from *S. panama* meningitis. There were no new positive stools subsequent to April 15, 1944, as far as the hospital was concerned. Stool specimens obtained from other members of households into which a *Salmonella*-positive infant had been introduced showed, however, that the infection spread readily to other members of the household, particularly among young brothers or sisters. In all, 72 individuals among other members of the household had *Salmonella*-positive stools. TABLE 2 shows the frequency with which positive isolations of *S. panama* were found among the other members of a household by size of the household.

In all, *S. panama* was isolated from 138 individuals, many of whom were apparently well at the time of our investigation. The 138 individuals with positive stools were distributed as follows: 20 newborn infants in the hospital; 1 adult hospitalized female; 1 staff graduate nurse; 1 staff nurse's aide; 1 staff floor maid; 2 hospitalized mothers of newborn infants; 1 adult staff male kitchen employee; 4 babies on the pediatrics service; 4 babies on the outpatient service; the 31 babies who had been born in the hospital and discharged as apparently well prior to March 10, 1944; and the 72 individuals among other members of the families into which an *S. panama*-positive infant had been introduced.

Because this study was undertaken in March 1944, and positive specimens were obtained from babies studied in all months from July 1943 through January 1944, an opportunity was afforded to study the length of time infants

TABLE 2

FREQUENCY OF POSITIVE ISOLATIONS OF *S. PANAMA* IN OTHER HOUSEHOLD MEMBERS BY SIZE OF HOUSEHOLDS

No. of families	No. of members in family	No. becoming *S. panama* +
13	3	1
9	5	1
8	4	1
6	4	2
3	6	1
2	5	3
1	4	3
1	5	2
1	6	2
1	6	4
1	7	1
1	8	1
1	9	1
1	9	4
1	11	3

might continue to remain positive for *S. panama*. It seems reasonable to hypothesize that the 31 babies who were found positive for *S. panama* in our survey of infants who had been born in the study hospital prior to March 10, 1944, were positive at the time of discharge. While this hypothesis seems reasonable, however, it is of course not based on laboratory-proved data. For this reason, TABLE 3 shows the number of infants remaining positive for *S. panama* by number of months, based both on the interval between first and last laboratory-proved positive specimens and on the interval between discharge from the hospital and the last laboratory-proved positive specimen.

TABLE 3

FECAL SPECIMENS REMAINING POSITIVE FOR *S. PANAMA* BY NUMBER OF MONTHS

Number of months	Based on interval between	
	First and last positive specimen	Discharge from hospital and last positive specimen
1	14	1
2	13	0
3	11	2
4	5	1
5	6	6
6	2	10
7	1	6
8	0	5
9	1	7
10	0	6
11	0	1
12	3	9
15	1	1
16	0	1
19	0	1

From this table, which involves 57 infants, it is obvious that very young infants are likely to have positive stools for a considerable period of time following clinical recovery from an *S. panama* infection. The date of the last positive isolation in this series was August 29, 1945, at which time *S. panama* disappeared from the community and has not reappeared in this particular community and has been isolated only infrequently elsewhere in Michigan.

Discussion

One might well wonder how *S. panama* infection could smolder in a community and in the maternity wing of a hospital from at least June 1943 to March 1944, a period of over eight months, without coming to the attention of the local or state health departments.

Special factors involved were: the outbreak occurred during the war years when communities were disrupted; the hospital administrator, who later resigned, was fearful of adverse publicity; mothers and infants were, for the most part, discharged on the third or fourth day post partum.

This last factor was probably the most significant one, since the incubation period of *S. panama* diarrhea from an average infective dose is 7 to 10 days, so that the infants were discharged as apparently well, only to develop diarrhea at home. Most of the diarrhea that did develop was not particularly severe and, for the most part, did not require hospitalization. Further, the three cases of *S. panama* meningitis that did develop at home were all hospitalized in hospitals other than the one in which they had been born and that was experiencing the outbreak of diarrhea.

It is, of course, impossible to determine arbitrarily just what the sequence of events was. The most probable explanation would be that a mother with an asymptomatic *S. panama* infection was admitted to the maternity wing and infected her newborn infant, who in turn infected hospital personnel; or the mother could have infected hospital personnel directly; or a new employee might have introduced the organism.

In any event, *S. panama* became established in the obstetrical unit and the stage was set for the tragedy of 18 deaths from *S. panama* meningitis.

Factors contributing to the spread of this enteric organism were: the nursery was overcrowded to the extent of twice its normal load; there was a decided shortage of trained nurses; there was excessive use of untrained personnel; and there were only enough trained supervisors to cover the daytime shift. All these factors, coupled with a hospital policy that prevented the obstetrical personnel from removing a sick infant from the nursery for newborn infants until the baby had been seen by the attending physician, resulted in a very heavy seeding of the organism among the other infants, hospital personnel, and others.

The dramatic concentration of cases and deaths in March 1944 was probably due to overwhelming infective doses of *S. panama*, with an attendant shortening of the incubation period. Certainly the deaths in March of two 5-day-old infants from *S. panama* meningitis would suggest this.

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ENTEROPATHOGENIC *ESCHERICHIA COLI* SEROTYPES

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Investigations in the past have made it clear that diarrheal diseases of infants may be caused by a variety of microorganisms. Epidemics and sporadic cases of infantile diarrhea or diarrhea of the newborn may be caused by agents such as *Salmonella* or *Shigella*. Occasionally, a bacterium of another group is thought to be the etiological agent in a particular outbreak and, in certain instances, viral agents have been incriminated. In a large number of otherwise unexplained epidemics of diarrheal disease of infants, however, certain *Escherichia coli* serotypes are recovered. The designation enteropathogenic *E. coli* has been suggested for these particular serotypes.

The concept that certain *E. coli* types might be involved in infantile diarrhea is not a new one. For more than 50 years workers have investigated and discussed the role of coliform bacteria in summer diarrhea, infantile enteritis, or diarrhea of the newborn (see Dupont[1] for references to earlier literature). Unfortunately, most of the earlier investigators were hampered by the fact that they used only biochemical tests in their attempts to differentiate between the suspected *E. coli* cultures isolated from cases and those recovered from normal infants or adults. By themselves, biochemical methods are inadequate for such differentiation because the *E. coli* group is composed of many different serotypes that often give identical biochemical reactions. A few workers used serological methods, however, in addition to biochemical tests. In fact, Goldschmidt[2] employed a slide agglutination technique to differentiate "Dyspepsiekoli" cultures from cases of infantile diarrhea and to trace the epidemic spread of the bacteria in the investigation of an institutional outbreak. As pointed out by Dupont,[1] it is unfortunate and strange that Goldschmidt's work did not receive the attention it deserved. Also, Dulaney and Michelson[3] utilized serologic methods to demonstrate the relationship of a number of strains of *Bacterium coli mutabile* which they isolated from cases of infantile diarrhea during an epidemic. Recently, it has been learned that the cultures isolated by Dulaney and Michelson belong to *E. coli* O group 18a, 18c.

The family Enterobacteriaceae is composed of several groups of bacteria such as the *Salmonella* group, the *Shigella* group, and the *Escherichia coli* group. Each of these groups is made up of biochemically similar microorganisms, and the groups correspond to genera in greater or lesser degree. It should be emphasized that while types within a group cannot be differentiated by biochemical tests, the biochemical reactions given by a culture are used to determine the group of Enterobacteriaceae to which it belongs. Serologic methods must then be employed to determine the serotype within a biochemical group.

Antigens of E. coli. The work of Kauffmann[4-7] and his collaborators made possible the development of a system for serologic typing of *E. coli* cultures and resulted in an antigenic schema in which the microorganisms may be classified. Interest in members of the *E. coli* group has been stimulated by the association

TABLE 1
ANTIGENS OF *ESCHERICHIA COLI*

O group antigens	K antigens			H antigens
	L	A	B	
135	30	26	21	40
	Total, 77			

of certain serotypes with diarrheal disease. As a result, the *E. coli* antigenic schema has been extended so that, at present, 135 O, 77 K, and 40 H antigens are now known (TABLE 1). Further information relative to these antigens may be found in Kauffmann,[7] and Edwards and Ewing.[8] The O antigens of the bacteria are the somatic antigens not inactivated by heat at 121° C. K antigens are somatic antigens that occur as envelopes or as capsules. These antigens inhibit the agglutination of living bacteria in O antisera, but this inhibitory effect is inactivated by heat at 100° C. or at 121° C. The H, or flagellar, antigens are also inactivated by heat at 100° C. Antisera for O antigens are prepared by injection of smooth cultures that have been heated at 100° C. for two and one-half hours, or at 121° C. for two hours, to inactivate their K and H antigens. In the preparation of K antisera, formalinized or living cultures of K forms, selected from platings, are used for injection into animals. Whenever possible, nonmotile strains should be used for K antiserum production. H antisera are produced with cultures of bacteria that have been passed through semisolid agar several times to enhance the development of H antigen. Detailed discussion of methods for the production and use of O, K, and H antisera may be found in the publications of Kauffmann,[7] and of Edwards and Ewing.[8]

The K antigens of *E. coli* cultures are a class composed of at least three varieties, designated L, A, and B (TABLE 2). All varieties occur as sheath, envelope, or capsular antigens that inhibit agglutination of living bacteria in O antisera. One of the most important differences between L and B antigens is that the antibody-binding power of L antigen is inactivated by heat at 100° C., whereas the binding power of B antigen is not. A pure L antiserum may be prepared by absorption of an OL antiserum with a heated suspension of the homologous strain. Pure B antiserum cannot be prepared by this procedure because the antibody binding power of B antigen is not inactivated by heat and, if OB antiserum is absorbed by a suspension of the homologous culture, both O and B agglutinins are removed from the antiserum.

Since complete serologic typing of *E. coli* cultures is a time-consuming and somewhat involved procedure, it may properly be considered a function of a few centers that are equipped for the work. The personnel of many laboratories may prefer to limit their activities to isolation and preliminary identification and to forward selected strains to a center equipped for further study.* It should be emphasized, however, that all laboratories should complete the

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* Methods and procedures that may be used for preliminary work were given by Ewing and Edwards.[9]

TABLE 2

THE K ANTIGENS OF *ESCHERICHIA COLI*

Variety of K antigen	Characteristics
L	1. Agglutinability of L antigen in L antisera inactivated by heat at 100° C., 1 hr. 2. Suspensions rendered agglutinable in O antiserum by heat at 100° C., 1 hr. 3. Antibody binding power inactivated by heat at 100° C., 1 hr. 4. Antigenicity inactivated by heat at 100° C., 1 hr. 5. Occur as envelope or sheath, occasionally as a capsule.
A	1. Agglutinability of A antigen in A antiserum inactivated by heat at 121° C., 2.5 hrs. 2. Suspensions rendered agglutinable in O antiserum by heat at 121° C., 2.5 hrs. 3. Antibody binding power not inactivated by heat at 100° C., 2.5 hrs. or at 121° C., 2 hrs. 4. Antigenicity inactivated by heat at 121° C., 2.5 hrs. 5. Occur as capsules.
B	1. Agglutinability of B antigen in B antiserum inactivated by heat at 100° C., 1 hr. 2. Suspensions rendered agglutinable in O antiserum by heat at 100° C., 1 hr. 3. Antibody binding power not inactivated by heat at 100° C., 1 or 2 hrs. or by 121° C., 2 hrs. 4. Antigenicity inactivated by heat at 100° C., 1 hr. 5. Occur as envelopes or sheaths.

following steps in the identification of *E. coli* types, whether the cultures are to be sent to a center or not: (1) determination of biochemical reactions; (2) slide agglutination tests with living bacterial suspensions in OB antisera; (3) slide agglutination tests with heated suspensions; and (4) titration of heated suspensions for confirmation of the O antigen group. These procedures do not permit serotype determination but they do allow identification of the O antigen group of those serotypes for which antisera may be available. It should be emphasized that anyone may enlarge the scope of his *E. coli* work as time and facilities permit. In fact, this procedure is to be commended and encouraged, for additional centers are needed.

Complete serotyping of *E. coli* cultures from cases of diarrheal disease is dependent upon the characterization of all of the antigenic components about which any information is available; that is, upon the determination of the O, K, and H antigens of the strains. The steps generally followed in the study of these components may be outlined as follows: (1) slide agglutination tests in which living suspensions are examined in both OB and O antisera prepared with those serotypes that have been associated with diarrheal disease (TABLE 3) and used for certain *sub judice* serotypes; (2) slide tests in which heated suspensions are tested in the same OB and O antisera; (3) titration of heated suspensions in serial dilutions of the indicated O antisera for confirmation of the O antigen group; (4) slide tests in absorbed factor antisera to differentiate related O antigen groups and to determine the component O antigenic fractions within an O antigen group; (5) titration of K (B) antigen suspensions in K (OB) antisera for confirmatory purposes; and (6) determination of H antigens.

In steps 1 and 2, antigen suspensions are tested in OB and O antisera in slide tests, first as living suspensions and again after heating. Agglutination of

TABLE 3

ESCHERICHIA COLI SEROTYPES ASSOCIATED WITH DIARRHEAL DISEASE

O antigen	K antigen	H antigens (and synonyms)
26	60 (B6)	NM* (E893), 11
55	59 (B5)	NM (β), 2, 4, 6, 7, 8, 10, 21
86	61 (B7)	NM (E990), 8, 9, 10, 11, 34
86	62 (L)	2
111	58 (B4)	NM (α), 2 (D433), 4, 11, 12, 16, 21
112a, 112c	66 (B11)	NM (Guanabara)
119	69 (B14)	NM, 6 (Aberdeen 537-52), 9, 18, 27 (W34)
124	72 (B17)	NM, 19, 30, 32
125a, 125b	70 (B15)	19 (Canioni), 21
125a, 125c	70	6
126	71 (B16)	NM, 2 (E611), 27
127a	63 (B8)	NM (Holcomb)
128a, 128b	67 (B12)	2 (Cigleris), 8, 9
128a, 128c	67	NM, 8, 10, 12

* Nonmotile.

TABLE 4

PRELIMINARY EXAMINATION OF THE K ANTIGENS OF *ESCHERICHIA COLI* CULTURES FROM CASES OF INFANTILE DIARRHEA (SLIDE TESTS)

Antigen suspensions	*E. coli* antisera							
	O26:B6	O26	O55:B5	O55	O111:B4	O111	O127:B8	O127
K antigen (living)	—	—	4	—	—	—	—	—
O antigen (100° C., 1 hr.)	—	—	4	4	—	—	—	—

Symbols: —, no reaction; 4, complete agglutination.

a living antigen in an OB antiserum and lack of a reaction in the corresponding O antiserum are indicative of the presence of B antigen in the strain. If such a reaction occurs in one of the OB antisera, the suspension is heated at 100° C. for one hour, cooled, and retested in the indicated OB and O antisera. If the culture belongs to the O antigen group, the heated antigen may be expected to react in both OB and O antisera. An example of results that may be anticipated with freshly isolated cultures is given in TABLE 4. It is to be emphasized that in slide agglutination tests with the unabsorbed antisera employed in steps 1 and 2, cognizance should be taken only of strong reactions, and undue significance should not be attached to weak and delayed agglutination. Also, it must be pointed out that if a living suspension reacts strongly in O, as well as in the OB antiserum, the culture must be plated, and colonies that are inagglutinable in O antiserum must be selected for reexamination.

For the third step, broth cultures that have been heated at 100° C. for one hour are titrated in serial dilutions of O antiserum. Such tests are read after 16 hours incubation in a water bath at 48 to 50° C. If the results of slide agglutination tests performed in the first steps are clear-cut and the culture is inagglutinable in O antisera, confirmation may be expected in the titrations. Other *E. coli* cultures, however, are related to certain of the serotypes associated with infantile diarrhea through possession of common O antigenic fractions. A strain that belongs to one of these related O groups may be expected to cross-react in the titrations. Also, it should be remembered that living suspensions of *E. coli* cultures that belong to related O antigen groups may react in OB and O antisera in slide tests if they are not O-inagglutinable, and heated suspensions may be expected to cross-react. As an example of this, the O antigens of O group 25 strains are related to those of O26, and an O group 25 suspension may react in O26:B6 and O26 antisera in slide tests, but when tested by titration in O26 antiserum, only a relatively low-titered reaction is obtained (TABLE 5). If a culture thought to belong to O group 26 reacts in this manner, the suspension is titrated in O group 25 antiserum.

In step 4, heated antigen suspensions are examined in absorbed O antisera by means of slide agglutination tests. These examinations serve one or the other of two purposes: (1) to differentiate the O antigens of a culture from those of strains that belong to related O antigen groups, as illustrated in TABLES 5 and 6; or (2) for the characterization of O antigenic components within a single O antigen group. For example, the two absorbed antisera listed in TABLE 7 may be used for this purpose. As will be mentioned later, these detailed analyses are necessary for accurate epidemiological studies. A list of absorbed O

TABLE 5

O ANTIGENIC RELATIONSHIP OF *ESCHERICHIA COLI* O25 AND O26

E. coli O antigen suspensions (100° C., 1 hr.)	*E. coli* antisera			
	O25		O26	
	Unabsorbed	Absorbed by O26	Unabsorbed	Absorbed by O25
O25	20,480*	10,240	1,280	0
O26	1,280	0†	20,480	20,480

* Reciprocal of highest dilution in which agglutination occurred.
† No reaction at 1:40, the lowest dilution tested.

TABLE 6

EXAMPLE OF ABSORBED *E. COLI* ANTISERA USED IN DETERMINATION OF O ANTIGEN GROUPS (SLIDE TESTS)

O antigen suspensions (100° C., 1 hr.)	*E. coli* O antisera			
	25		26	
	Unabsorbed	Absorbed by 4, 18, 26, 102	Unabsorbed	Absorbed by 4, 18, 25
4	+	−	+	−
18	+	−	+	−
25	+	+	+	−
26	+	−	+	+
102	+	−	+	−

TABLE 7

THE O ANTIGENIC COMPONENTS OF *E. COLI* O GROUP 128

E. coli O antigen suspensions (100° C., 1 hr.)	*E. coli* O antisera			
	128a, 128b		128a, 128c	
	Unabsorbed	Absorbed by 128a, 128c	Unabsorbed	Absorbed by 128a, 128b
128a, 128b	10,240	1,280	2,560	0
128a, 128c	10,240	0	5,120	1,280

TABLE 8

ABSORBED *E. COLI* O ANTISERA REQUIRED FOR DIFFERENTIATION OF SEROTYPES ASSOCIATED WITH DIARRHEAL DISEASE

E. coli O antiserum	Absorbed by suspensions of *E. coli* O group(s)
25	4, 18, 26, and 102
26	4, 18, and 25
86a, 86b	86a and 90
86a	90
127a	90
127a, 127b	90 and 127a
111a, 111b	111a, 111c
111a, 111c	111a, 111b
112a, 112b	112a, 112c
112a, 112c	112a, 112b
119	48
125a, 125b	73
125a, 125b	125a, 125c
125a, 125c	125a, 125b
126	75
75	126
128a, 128b	87
87	128a, 128b
48	128a, 128b and 87
128a, 128b	128a, 128c
128a, 128c	128a, 128b

antisera known to be required for use with *E. coli* cultures from cases of diarrheal disease is given in TABLE 8.

In step 5, the results of slide agglutination tests with living antigens (step 1) are confirmed by titration of formalinized broth cultures in the indicated OB antiserum. Antigen suspensions employed in such tests generally are prepared by inoculation of broth from isolated colonies selected for O inagglutinability. After incubation for four to five hours the cultures are formalinized by the addition of 0.5 per cent formalin. The O inagglutinability of the antigen suspension is controlled by titration of the same formalinized antigen in serial dilutions of O antiserum. Both in the test in OB antiserum and the control titration are placed in an incubator at 37° C. for two hours and then removed to a refrigerator for overnight incubation. Characteristic agglutination (which results in the formation of compact membranes at the bottom of the tubes) to the B titer of the OB antiserum (usually 1:320 or 1:640) and lack of a reaction in O antiserum serves to confirm the identity of the B antigen in most instances. A slight, incomplete reaction in low dilutions (<1:160) of O antiserum is acceptable, but if complete agglutination is seen, even in low dilutions, the results are considered equivocal and the tests must be repeated with antigen suspensions prepared from newly selected colonies. In some instances where circumstances warrant, B antigens are determined by absorption of OB antiserum with a heated (100° C., two hrs.) suspension of the culture under examination. If the culture possesses the same O and B antigens possessed by the strain used to prepare the OB antiserum, both O and B agglutinins are removed from the antiserum.

The final, or sixth, step in serologic typing is determination of the H antigens of motile strains. Freshly isolated cultures usually are poorly motile, and antigens prepared from them do not react in a characteristic manner when tested in H antisera. To promote motility and enhance H antigen development, cultures are passed through semisolid agar medium several times, after which broth cultures are prepared. Following incubation, these cultures are formalinized and used as antigens in H agglutination tests. Such antigens are first tested in eight pools, each of which contains 10 H antisera. These are single-tube tests. The final dilution of the individual antisera in each pool is 1:1000. The tests are incubated in a water bath at 48 to 50° C. and are read after 15, 30, and 60 minutes. If H agglutination occurs in one of the pools, the antigen is then tested in each of the component antisera of that pool by means of single-tube tests at 1:1000 dilution. The results so obtained are confirmed by titration in serial dilutions of antisera. With few exceptions, the known H antigens of *E. coli* cultures do not cross-react when tested in the antiserum dilutions mentioned. Strong reciprocal relationships, however, are known to exist between H antigens 1 and 12, 8 and 40, 11 and 21, and 30 and 32. For this reason, antisera for these H antigens are paired in the pools mentioned above, and cross-absorbed antisera are used in final identification.

The H antigens of many *E. coli* cultures may be determined by means of slide agglutination tests using absorbed H antisera. The advantage of such examinations lies in the fact that absorbed H antisera may be used in low dilutions (1:10 or 1:20) and the H antigens of many sluggishly motile cultures can be determined without passage in semisolid medium, or after only one or two passages. Thus far, pure H antisera have been prepared for all of the H antigens listed in TABLE 3 and for a few additional H antigens. The remainder are under investigation.

If *E. coli* cultures do not belong to any of the serotypes presently known to be associated with infantile diarrhea and cannot be serotyped by means of the procedures outlined above, it is necessary to resort to other methods for O group and K antigen determinations.

Heated O antigen suspensions prepared from such cultures are first tested in several pools that contain O antisera for 135 O antigen groups. Each O antiserum pool contains from 10 to 13 individual antisera that are grouped together according to antigenic relationships or for convenience. The pools are diluted in such a way that the concentration of each component antiserum is 1:100. They are used in single-tube tests in which the final dilution is 1:1000. If agglutination occurs in one of the O antiserum pools, the O antigen preparation is then tested in each of the antisera contained in the pool. These are also single-tube tests in which antiserum dilutions of 1:1000 are used. This step is followed by titration of O antigen suspensions in the antiserum, or antisera, indicated by the single-tube tests. Since there are many reciprocal relationships between O antigen groups, it often is necessary to titrate an O antigen preparation in two or more individual O antisera. Furthermore, antisera absorbed by one or more heterologous O groups are required for O group identification in many instances. These absorbed O antisera are used in slide tests after the results of titrations are known.

For determination of the K antigens of *E. coli* strains that belong to other serotypes, living suspensions are tested in pooled K antisera on slides. Each pool contains five antisera and the dilution of the individual antisera in each is 1:5 or 1:10. Subsequently, the K antigen suspension is tested in the individual antisera contained in the pool, or pools, in which a reaction has been obtained. Then, the degree of inagglutinability of the antigen is determined by tests in O group antiserum, and the results of slide tests are confirmed as outlined in step 5.

The H antigens of serotypes other than those presently associated with diarrheal disease are determined in the manner given in step 6.

There are several reasons why complete and detailed serologic analysis of *E. coli* strains is considered desirable. First, complete serotyping is necessary for accurate epidemiological studies, since different serotypes exist within the several O antigen groups known to be associated with diarrheal disease. In attempts to trace the source of infection in an outbreak or in studies of intrahospital and interhospital spread of infection, determination of O antigenic components and of H antigens of the strains involved would seem to be essential, just as it is in the investigation of salmonellosis. These determinations also are necessary for the detection of other serotypes of the same O antigen group that may be introduced into a ward by newly admitted cases during the course of an epidemic. The reader is referred to the work of Wright, Roden, and Villanueva[14] for an excellent illustration of the value of H antigen determinations in epidemiological studies.

Second, complete serotyping is necessary for the compilation of accurate data regarding incidence of various serotypes. Within O group 55 there are several serotypes, of which O55:B5:H6 appears to be most common in the United States. Of the several serotypes contained in O group 128, serotype O128a, 128b:B12:H2 is of more common occurrence.[33] Further studies on serotypes within these and other O antigen groups should lead to the accumulation of much more accurate information on incidence and geographic distribution of serotypes associated with infantile diarrhea.

Third, complete antigenic characterization is required for study of serotypes that may be suspected of being of etiological significance in diarrheal disease, for no given serotype can be definitely incriminated until it has been fully characterized and found associated with outbreaks of disease in widely sepa-

TABLE 9

SEROLOGICALLY RELATED *E. COLI* CULTURES THAT ARE EPIDEMIOLOGICALLY DISTINCT

O	K	H	Sources	References
86a	61 (B7)	d*	Infantile diarrhea	Charter & Taylor,[16, 17] Ørskov,[18] Ewing *et al.*[19]
86a, 86b	64 (B9)	35	Normal adult	Ewing *et al.*[19]
111a, 111b	58 (B4)	d	Infantile diarrhea	Bray,[20] Bray *et al.*[21] Varela *et al.*,[22] Taylor[23]
111a, 111c	58 (B4)	NM	Monkeys	Ewing *et al.*[35]
127a	63 (B8)	NM	Infantile diarrhea	Ewing *et al.*[19]
127a, 127b	65 (B10)	4	Normal children	Ewing *et. al.*[19]

* An epidemic strain may be nonmotile or may possess one of several H antigens.

rated areas. Parenthetically, it may be added that strains of *E. coli* employed in pathogenicity tests should be characterized as completely as possible.

As a fourth reason, it may be said that detailed studies on the antigenic components of *E. coli* cultures often are of considerable value in the differentiation of serologically related strains that are epidemiologically distinct. Cultures of alleged "infantile diarrhea types" isolated from such diverse sources as normal persons and animals should be subjected to the closest scrutiny because careful examination often reveals that such strains are not infantile diarrhea serotypes but only related types. Several instances in which such differentiation has been possible are given in TABLE 9. In addition, Ørskov[18] has shown that cultures of *E. coli* O group 86 isolated from urine, from appendicitis, and from bovine mastitis differ in their H and K antigen content from strains of this O antigen group isolated from infantile enteritis. Also, Ørskov and Fey[34] demonstrated that a culture of O group 55 recovered from bull semen contained an O antigen factor not present in strains from cases of diarrheal disease in infants.

In concluding, we may add that complete serotyping of *E. coli* cultures has its own intrinsic values. It aids in the classification of many so-called "paracolon *Escherichia*" cultures, which previously could be classified only by biochemical means. Also, studies of *E. coli* serotypes and their relationships to members of other groups of Enterobacteriaceae have contributed to the formulation of the present day conception of the enteric family as a large interrelated group of bacteria. Last, complete serotyping and research are urged because the resultant data add to our limited knowledge of the very large *E. coli* group.

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EXPERIMENTAL DIARRHEAL DISEASE OF HUMAN VOLUNTEERS DUE TO *ESCHERICHIA COLI*

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Usually, in the present era of microbiological science, the finding of a suspected agent of disease is followed fairly rapidly with confirmatory evidence of its significance. Occasionally the question of the role of an organism, relative to a syndrome or disease, will remain unsettled for years. Such has been the case with certain serotypes of *Escherichia coli* reported in infant diarrhea by numerous investigators during the last decade.

Unusual difficulties have been encountered in resolving the question of the etiology of these organisms. First, there has been the very real problem of overcoming the concepts of the past in regard to the commensal role of all coliform organisms in the intestinal tract of man. Second, investigators have been denied the aid of the agglutination reaction with which Shiga and other early workers clinched the pathogenic significance of bacillary dysentery organisms. Infants have either developed no reaction or a weak rise in serum agglutinins when infected with enteropathogenic *E. coli*. Third, there has been no experimental animal for determining the pathogenicity of diarrheal types of *E. coli* and for contrasting their disease-producing properties with those of normal coliform bacteria. Of necessity, the case for the etiologic role of *E. coli* organisms in infant diarrhea has depended upon extensive epidemiologic, clinical, and bacteriologic observations.

In the absence of a susceptible experimental animal, it has been natural to resort to man as a test subject. A limited number of investigations have been made in which human adult volunteers, or infants, have been fed *E. coli* serotypes 55:B5, 111:B4, or 127:B8. The evidence furnished by these studies is not completely satisfying but, in general, it tends to confirm clinical and epidemiologic observations regarding pathogenicity. The present paper is a review of these studies.

Kirby, Hall, and Coackley,[1] in England in 1950, were the first to report human feeding experiments with any of the enteropathogenic *E. coli*. They used a total of 18 adult volunteers and employed two strains of *E. coli* 111:B4. Both test strain A and strain B were derived from the same stock culture which had been refrigerated on an agar slope since its isolation 11 months previously. Strain A was kept for long intervals at room temperature on agar plates, while strain B was a recent transfer from the old stock culture. Both organisms were grown under 10 per cent CO_2-enriched atmosphere before harvesting. The dose of test organisms employed was approximately 2 billion (2×10^9).

The history of the test strains employed is particularly pertinent because strain A produced illness in four of six volunteers who ingested it, while strain B caused no symptoms in the three men who were fed it. Illness in those who ingested strain A was mild, amounting to diarrhea and abdominal discomfort in three men, and diarrhea, vomiting, and abdominal discomfort in a fourth man. On the average, symptoms developed in 12 hours and disappeared in 24 hours. Controls consisted of three men who ingested uninoculated medium in milk—the vehicle for test feedings—and six men who ingested two different strains of fecal *E. coli* unrelated serologically to type 111:B4. No control became ill. Only three of those who ingested strain A and the three who ingested strain B were tested for a rise in specific agglutinins. In all instances the titers were less than 1 in 10.

Kirby, Hall, and Coackley[1] reported that they were investigating possible differences in strains A and B, but to date nothing has appeared in the literature concerning their findings.

The experiment of Neter and Shumway[2] on a two-month-old infant with congenital defects is the only instance of an infant-feeding study carried out with an enteropathogenic *E. coli* in this country. The infant, shown previously not to be harboring *E. coli* 111:B4, was fed approximately 100 million 111:B4 organisms in formula. Diarrhea and weight loss were produced within 24 hours. The bacteria were demonstrated within 48 hours in very large numbers in the throat, nasopharynx, and feces. Treatment with Terramycin resulted in clinical improvement and disappearance of infecting organisms within two days. Upon recovery, the infant was fed a different type of *E. coli* recovered from its feces prior to contact with type 111:B4. No diarrhea or weight loss followed this event.

The next study that will be summarized is out of place in this review from the standpoint of chronology, but its inclusion at this point is obvious since it deals with infant-feeding experiments. This work was reported at the Sixth International Congress of Microbiology in Rome, Italy, in 1953 by H. Hiroki[3] of the Kitasato Institute for Infectious Disease in Tokyo, Japan. The full report is not available in published form and exists only in manuscripts distributed by Hiroki. This work has already been referred to in the literature by Dupont[4] of Copenhagen, Denmark.

In a modern nursery containing 20 babies, an outbreak of diarrhea and vomiting apparently was started by the introduction of two diarrheal infants whose stools subsequently yielded *E. coli* 55:B5. A total of 15 babies in the nursery was soon found to be infected with this type of pathogen. Nine of the infants developed diarrhea and vomiting. The other six passed a few soft stools but otherwise remained well. After the start of the outbreak, three of the well infants, ages 10 and 11 months, were fed *E. coli* 111:B4 (the dosage is not stated definitely). Two of these infants developed diarrhea and excreted the test organisms, and the third infant showed no symptoms other than soft stools. Within three weeks, 10 out of 22 babies in the nursery had developed infection with *E. coli* 111:B4 while, at 4 weeks, 18 out of 22 babies excreted either type 55:B5 or 111:B4.

Obviously, conditions for a feeding experiment were not optimal in a nursery undergoing an outbreak. It seems clear, however, that two of three well babies developed diarrhea following ingestion of 111:B4 organisms. The spread of this coli type among other infants in the nursery is of interest but of limited significance in the absence of detailed clinical information. The same spread might have occurred had a normal fecal strain of *E. coli* been introduced into this population. Use of the word "infection" by this investigator implies only the establishment of enteropathogenic *E. coli* in the intestinal tract.

Two reinfection experiments reported by Hiroki are of considerable interest: (1) An infant who had natural infection with 55:B5 *E. coli*, with symptoms of diarrhea, excreted the organism for 15 days. After a period of 17 days in which the infant was well, a feeding of the homologous strain of 55:B5 was made. Again the baby developed diarrhea, but in milder form. Excretion of 55:B5 organisms lasted for 34 days.

(2) A second infant who developed symptoms following feeding with 111:B4 organisms was reinfected with a second feeding of the homologous strain of 111:B4 after apparently becoming free of infection. Again symptoms developed, but of a milder form. This baby excreted the organisms for 44 days. Hiroki points out that little protection was conferred by the first infection, and excretion of infecting organisms appeared to be in excess of the quantity seen during the original illness.

In a further experiment, Hiroki[3] collected feces from an infant discharging both 55:B5 and 111:B4 organisms, and from a second infant excreting type 111:B4 only. These he filtered through paper and, in addition, centrifuged and Seitz-filtered a portion of each. He fed the portions bearing organisms and the Seitz-filtered portions to different groups of babies, and produced diarrhea in the former groups and no symptoms in the latter. His experiment can, of course, be criticized because the Seitz filter might very well have retained most, if not all, of any viral agent present. This is the first experiment, to my knowledge, in which an attempt has been made to transmit a possible viral agent by an infant-feeding experiment with stool inoculum of the type described.

In 1951 in this country, human adult feeding experiments were planned by Ferguson and June[5] before the authors were aware of the work of Kirby, Hall, and Coackley. When it was learned of the considerably different results obtained by the British investigators in feeding two different strains of *E. coli* 111:B4, it was decided to feed a mixture of three enteropathogenic *coli* strains. The test strains selected were isolated locally, and all three had been confirmed as type 111:B4 by Kauffmann. A total of 114 adult healthy males was used, divided into 56 men in the control groups and 58 in the test groups. Control personnel received milk only, with no knowledge of the innocuous nature of the drink, while test personnel received milk seeded with organisms. The first group of test personnel ingested 9 billion *E. coli* 111:B4 organisms, the second 6.5 billion, the third 530.5 million, and the fourth group 7 million organisms. Feeding 530.5 million organisms or less produced a mild illness or no effect, but feeding 6.5 or 9 billion organisms produced symptoms varying from severe to slight in all volunteers. A typical severe case experienced abdominal discomfort and sometimes nausea within 4 to 6 hours after the feeding, followed by severe diarrhea within 9 to 12 hours. Fatigue and cramps with diarrhea were usual within the first 24-hour period, and symptoms sometimes persisted for as long as 5 to 6 days. A rise in temperature was usual within the first 12 hours after feeding.

A group of 11 men, each of whom ingested 9 billion organisms of a suspension of fecal *E. coli* serologically unrelated to known enteropathogenic *E. coli*, experienced no ill effects and developed no serum agglutinins to this organism. Control personnel experienced no illness beyond a few complaints of cramps or headache, and none developed a rise in serum agglutinins for *E. coli* 111:B4. In contrast, 38 of 58 men in the test groups showed a rise in serum agglutinins for *E. coli* 111:B4, regardless of whether symptoms had been present or not.

In 1952, experiments in feeding adult volunteers with *E. coli* 55:B5 organisms were carried out by June, Ferguson, and Worfel[6] that followed fairly closely the plan of the experiments just summarized. A total of 71 men was used, 31 of whom ingested living cultures of *E. coli* 55:B5 of a dosage varying from 16 billion to 143 million organisms. Eight men were also each fed a dose of 16 billion killed organisms of type 55:B5, while an additional eight volunteers each ingested a dose of 15 billion organisms of a "normal" fecal *E. coli*. Control personnel received uninoculated milk.

Symptoms in those who ingested living 55:B5 *E. coli* were generally milder than those exhibited by volunteers fed the 111:B4 organisms. Below a level of 1.725 billion organisms the symptom response was slight. In higher dosage groups, however, there was one case of severe response and four cases of moderate illness. Volunteers who ingested either killed 55:B5 organisms or living "normal" fecal *E. coli* organisms experienced no illness. Specific agglutinin response to the organism fed was present among the majority of those men who ingested living 55:B5 organisms, but it was not found among the men who received killed 55:B5 cultures or the "normal" *E. coli*. It was felt that the lack of serologic response in the latter two groups answered to some degree the valid objection that massive doses of living 55:B5 cultures might elicit a response similar to that of a killed vaccine.

The specificity of serologic reactions in the foregoing O55 experiments was confirmed in the laboratories of Erwin Neter by hemagglutination tests.[7] All available sera were identified to him by number only until his results were completed. It was found that, except for the much greater sensitivity of the hemagglutination tests, Neter's results paralleled those obtained by June, Ferguson, and Worfel.[6]

In Japan, the feeding experiments of Fukumi and Kosakai[8] on adult volunteers agree in some respects with results obtained in this country and disagree in others. The Japanese workers fed a mixture of 111:B4 strains in a dosage of 5 billion organisms to eight volunteers and produced diarrhea in six individuals and other symptoms in the remaining two. They were not able to demonstrate an agglutinin response after the feeding, a surprising result in consideration of the large number of organisms ingested. Volunteers fed a "normal" human fecal strain of *E. coli* showed no symptom response, but three of five persons who ingested an *E. coli* culture isolated from a monkey developed mild diarrhea. It is relevant at this time to point out that selection of "normal" fecal *E. coli* strains is a gamble, since we know nothing of the properties that make one strain harmless, but another enteropathogenic when fed to a subject.

The following experiment, carried out recently in this country by Wentworth, Brock, Stulberg, and Page,[9] illustrates the variation in individual susceptibility that one has come to expect in feeding studies. Two adult males, ages 28 and

32, ingested a mixture of *E. coli* 127:B8 cultures consisting of a dose of 4 million organisms for one man and 8 million organisms for the other. When compared with the dosage of 111:B4 and 55:B5 types that other workers have found necessary to produce symptoms, 4 million or 8 million organisms is very small. Nevertheless, both men experienced diarrhea, abdominal cramps, nausea, and mild-to-moderate malaise. The man who ingested only 4 million organisms experienced the more severe illness. By bacterial agglutination and hemagglutination tests, both volunteers were shown to develop a rise in specific antibody. Thirty-two days after the initial feeding took place the individual with milder symptoms ingested a second dose of 127:B8 cultures, this time a total of 8 million organisms. No symptoms of any nature were experienced.

Perhaps the most unusual experiments undertaken on human volunteers with any of the enteropathogenic *E. coli* are those of Koya *et al.*[10, 11] in Japan. By means of a double-lumen Miller-Abbott tube and fluoroscopic guidance, these workers took normal drainage specimens from three healthy adult male volunteers and found the duodenum, jejunum, and upper ileum practically sterile. Feeding the men with *E. coli* 111:B4 cultures in doses up to 1.4 billion organisms produced diarrhea, accompanied by nausea and vomiting. The fecal flora was largely taken over by 111:B4 cultures. A series of drainage specimens taken at intervals after the feeding yielded pure cultures, or nearly pure cultures, of 111:B4, from the small intestine. A fourth volunteer who was fed the smallest dosage of organisms, numbering approximately 27 million, developed no symptoms. Drainage specimens from this man yielded no *E. coli* 111:B4 cultures, and the organisms were not recovered in the feces up to four days after feeding. When this volunteer later was fed 1.6 billion 111:B4 organisms, however, he developed symptoms, and drainage specimens from the ileum gave a high count of type 111:B4. Koya and his colleagues made the assumption that proliferation of the organisms in the small intestine was the cause of illness in the volunteers, and that appearance of the cultures in the large intestine was of incidental significance.

In order to test the validity of this assumption, the ascending colon of each of four adult male volunteers was inoculated with 6 to 18 billion 111:B4 bacteria by means of the Miller-Abbott tube. No symptoms developed in the volunteers. Furthermore, the 111:B4 organisms could not be recovered from the stools of three men. Stools of a fourth volunteer yielded 111:B4 cultures only for 24 hours after inoculation.

As a further test, five more volunteers were inoculated with 111:B4 cultures in dosages up to 35 billion organisms, this time by means of sigmoidoscope and catheter. Inoculum was deposited up to the end of the descending colon after saline enema. No symptoms developed in these men, although two individuals excreted 111:B4 organisms for 7 to 10 days thereafter.

The very recent work of Scott Thompson[12] in England lends some support to the assumptions of Koya *et al.* Thompson did quantitative bacteriological studies of duodenal drainage specimens taken from a large number of infants who were either (1) normal, (2) ill with gastroenteritis due to enteropathogenic *E. coli*, (3) convalescing from coliform gastroenteritis or, (4) ill with gastroenteritis not due to enteropathogenic *E. coli*. In agreement with other workers, Thompson found that the normal infant had few or no *E. coli* in the duodenum. In infants of the second category he found that drainage specimens showed a high count of type 111:B4, 55:B5, or 26:B6 *E. coli*. In only one of five infants convalescing from gastroenteritis but still excreting enteropathogenic *E. coli* could he find *E. coli* organisms in drainage specimens. These were not of the enteropathogenic type. Finally, in cases of gastroenteritis not due to enteropathogenic *E. coli*, Thompson found only a few or no *E. coli* in duodenal drainage specimens. He feels that multiplication of enteropathogenic *E. coli* may take place throughout the whole length of the gut in infants infected with these organisms, but he places particular emphasis on their occurrence in the duodenum. Thompson asserts that clinically, pathologically, and bacteriologically there are striking resemblances between Asiatic cholera and gastroenteritis of babies due to enteropathogenic *E. coli*.

In the introduction to the study just summarized, Thompson[12] has pointed out that feeding tests of adults with enteropathogenic *E. coli* can scarcely be considered fair tests because of the large numbers of bacteria ingested. He considers that such feedings do not correspond to invasion of the body by a small infecting dose that becomes established and multiplies. It seems unlikely that Thompson will meet with any argument in this view, for there is no reason to believe that investigators who fed *E. coli* to adults were under the illusion that the tests approximated natural infection, particularly in the matter of dosage. It should be realized that Thompson's criticism also applies to human feeding tests with *Shigella*[13] and *Salmonella*,[14] in which the effective doses fed to volunteers were probably far in excess of the dose in natural infection.

Taken as isolated experiments, the adult feeding studies summarized in this paper would be of questionable significance but, taken as complementary studies to epidemiologic and clinical observations on coliform diarrhea, they have meaning. They give added weight to the view that enteropathogenic *E. coli*, and particularly types 111:B4, 55:B5 and 127:B8, are different from normal fecal *E. coli*. The organisms were toxic to adults when ingested in large numbers, whereas comparable doses of normal human fecal *E. coli* produced no effect. They produced a rise in agglutinins in adults when ingested that normal *E. coli* did not. Heated 55:B5 organisms produced no symptoms and no rise in serum agglutinins, suggesting that toxic components had been altered or destroyed. Fukumi[15] states in recent correspondence, however, that he has been able to produce diarrhea in some subjects by feeding large doses of heated type 111:B4, but the details of his experiment are not yet available.

Infant-feeding experiments, although open to criticism from the standpoint of heavy dosage, have clearly shown that symptoms similar to those of natural infection can be produced. Moreover, Neter and Shumway[2] demonstrated that prompt treatment with an antibiotic following artificial infection cleared up both symptoms and infecting organisms. A second feeding with a normal fecal *E. coli* produced no effect. The repeat feeding experiments of Hiroki,[3] on the other hand, produced reinfection with enteropathogenic *coli*.

From the experiments of Koya *et al.*[10, 11] and the observations of Thompson,[12] it would appear that certain of the enteropathogenic *E. coli* are able to adapt themselves and to multiply in the small intestinal tract of artificially infected adults or in naturally infected infants. Ordinary *E. coli* of the gut are apparently unable to do this. This combination of circumstances suggests several interesting possibilities: (1) the pathogenic effects of special *E. coli* types are exerted chiefly in the small intestine of man; (2) the ability of these organisms to grow in the small intestine of man may have more to do with their pathogenic properties than any special endotoxicity; or (3) a combination of special endotoxicity and ability to grow in the small intestine may constitute pathogenicity. These are, it must be stressed, implications only of the work of Koya and Thompson. Whether they will prove valid remains to be seen.

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ESCHERICHIA COLI ASSOCIATED WITH INFANTILE DIARRHEA*

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Reports on *Escherichia coli* and its relation to acute diarrhea in infants date from the study by Adam[1] in 1927 in Germany, in which he found certain fermentative types associated with diarrhea in infants. Fermentative and other biochemical reactions were, however, inadequate for demonstration of an etiologic relationship. In 1944 and 1947, Kauffmann,[2, 3, 4] in Denmark, published an antigenic schema for the *E. coli* group, based upon his earlier work and that of his collaborators, Knipschildt[5, 6, 7] and Vahlne.[8] In 1945, Bray,[9] in England, utilizing the antigenic schema of Kauffmann, was the first to demonstrate the association of a specific serologic type of *E. coli* with an epidemic of diarrhea among newborn infants, and focused attention more strongly on *E. coli* as a possible etiologic agent. This particular type of *E. coli* was shown subsequently to be *E. coli* O111:B4. Giles, Sangster, and Smith,[10] in 1949, in Scotland, reported a second serotype, O55:B5, associated with an outbreak of diarrhea among infants. Since these two serotypes of *E. coli* were discovered, and their association with infantile diarrhea reported, they have been isolated from infant patients in outbreaks of epidemic diarrhea in many parts of the world.

In the United States, bacteriological and clinical studies with certain serotypes of *E. coli* associated with diarrhea in infants date from 1950 and have been made, until recently, primarily by W. H. Ewing,[11-14] Erwin Neter,[15-21] William W. Ferguson[22-25] and their collaborators. In 1954, Stulberg, Zuelzer, and Nolke[26] reported the results of their study of an epidemic of diarrhea of the newborn in Detroit, Mich., caused by *E. coli* O111:B4; Wheeler and Wainerman[27] in Columbus, Ohio, used chloramphenicol and neomycin in the treatment and control of epidemic infantile diarrhea due to *E. coli* O111:B4; and Graber and Dunlop[28] reported on the incidence of serologic types of *E. coli* associated with infantile diarrhea among pediatric patients in the Denver, Colo., area. Also in 1954, methods for the isolation and preliminary identification of *E. coli* serotypes associated with cases of diarrhea of the newborn were published by Ewing and Edwards.[29]

The year 1955 has been unique in that two new serotypes of *E. coli* have been reported. Ewing, Galton, and Tanner[30] reported the detection of *E. coli* O111a, 111c:B4, isolated from monkeys involved in an epidemic of diarrhea. The second new serotype, identified by Ewing, Tanner, and Tatum[31] as *E. coli* O127:B8, was detected in epidemics of diarrhea in young infants in Cincinnati, Ohio, in the winter of 1953–54, by Cooper, Walters, and Keller;[32, 33] in Detroit, Mich., in 1954, by Stulberg, Zuelzer, Nolke, and Thompson;[34] and in Denver, Colo., in late 1954, by Bernet, Graber, and Anthony.[35]

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In this paper we shall deal with two phases of our *E. coli* studies: first, presenting information regarding the epidemic of diarrhea in the Children's Hospital, Cincinnati, Ohio, in which the new serotype O127:B8 was detected; and, second, presenting information regarding bacteriological studies of rectal swab cultures on patients in the Children's Hospital during the 19 months since our epidemic, in which search was made for 9 serotypes of *E. coli* as well as *Shigella* and *Salmonella*.

Between November 1953, and March 1954, there occurred, on our two infants' wards, an unusual incidence of diarrhea that progressed and spread regardless of the therapeutic use of various antibiotics and sulfonamides. The fact that the majority of the patients were infants, some newborn premature babies and some newborn full-term babies, and that repeated bacteriological study of rectal swab specimens failed to reveal the presence of *Shigella* or *Salmonella*, led us to consider the possibility that we were encountering an epidemic of diarrhea of the newborn, possibly due to one of the specific serotypes of *E. coli* previously found to be associated with diarrhea. The epidemic aspect was emphasized by the fact that a few infants admitted for some condition other than diarrhea developed the same diarrheal disease a few days after admission. Prior to the time of realization of the presence of an epidemic, rectal swabs were obtained for culture only from patients with diarrhea. Thereafter, rectal swabs were obtained from all infants on the two involved wards, regardless of the presence or absence of diarrhea, and also from all attending personnel in order to detect inapparent cases and carriers.

Methods

Stool specimens were obtained by using rectal swabs that were placed immediately in test tubes containing 2.0 ml. of sterile glycerin preservative solution.[36] These swabs were cultured on sorbitol agar* (Difco), MacConkey agar (Difco), and blood agar prepared with trypticase soy agar (BBL)† plus 5 per cent fresh defibrinated rabbit blood. In addition, the same swabs were cultured in the bacteriology laboratory of the Children's Hospital for *Shigella* and *Salmonella* on SS agar (Difco), MacConkey agar (Difco), brilliant green agar (Difco), and in tetrathionate broth base (Difco) which, after incubation overnight, was subcultured onto the three media.

The cultures studied in the bacteriology laboratory of the hospital failed, with few exceptions, to reveal the presence of any intestinal pathogens. The cultures studied in our research laboratory revealed, in acutely ill patients, a predominance of a type of *E. coli* differing from the *E. coli* usually found, in that it failed to ferment sorbitol, was a weak producer of indole, and did produce colonies on sorbitol agar resembling colonies of *E. coli* O111:B4 and O55:B5, but was not agglutinated in antisera specific for these two types of pathogenic *E. coli*. As the patients convalesced, this unusual type of *E. coli* gradually decreased in numbers, and the normal coliform bacteria became predominant.

It soon became evident that the sorbitol agar was much superior to blood agar and MacConkey agar for recognition of colonies of this unusual *E. coli* and, as the number of specimens increased, we began to rely entirely on the sorbitol agar for detection of its colonies. This medium is prepared by the Difco Laboratories with some modifications of the formula of Rappaport and Henig,[37] who substituted D-sorbitol for lactose in MacConkey agar, thus taking advantage of the fact that *E. coli* O111:B4 and O55:B5 ferment sorbitol late or not at all. Since the strain of *E. coli* we were finding also ferments sorbitol late (after 13 25 days) or not at all, this medium was ideal for our use. Strains of *E. coli* usually found in stool cultures produce red colonies on this medium after 18 to 24 hours incubation at 37°C. while strains of *E. coli* O111:B4, O55:B5, and O127:B8 produce colorless colonies. Nonfermenting colonies on sorbitol agar were picked and inoculated into tubes of sorbitol iron agar,* making stab and surface inoculations. This medium was also prepared by the Difco Laboratories following the earlier studies by Rappaport and Henig.[37] After 18 to 24 hours incubation at 37°C., the growth from sorbitol-nonfermenting colonies either failed to change the appearance of the sorbitol iron agar medium or produced a slight decolorization in the butt of the tube, while sorbitol-fermenting colonies produced acid, or acid and gas, in the butt. Cultures that did not ferment sorbitol were then submitted to slide agglutination tests and inoculated into a variety of media for determination of their biochemical characteristics after incubation at 37°C. It is possible that colonies of other enteric species that do not ferment sorbitol rapidly may be picked. In our laboratory, a few colonies of sorbitol-nonfermenting microorganisms were subsequently identified as *Shigella*, *Salmonella*, or *Paracolon*.

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* We are indebted to C. W. Christensen of the Difco Laboratories, Detroit, Mich. for supplying us generously with this medium.

† Baltimore Biological Laboratory, Baltimore, Md.

Serological antigenic classification of the strains of *E. coli* isolated from our patients were made possible through the courtesy of W. H. Ewing.† Early in our study, the strains of *E. coli* that we were detecting and that did not agglutinate in antisera specific for *E. coli* O111:B4 and O55:B5, were forwarded to Ewing for serological study. It was Ewing's conclusion‡ that our *E. coli* was a particular serotype with a new O antigenic group that most likely would be designated by F. Kauffmann as O127. Kauffmann's subsequent study confirmed the earlier opinion of Ewing. Further studies by Ewing revealed that the B antigen was not identical with, or significantly related to, any described *E. coli* B antigen. This new B antigen was designated B8, thus the designation *E. coli* O127:B8.

Diagnostic antisera. In the meantime, we prepared antiserum for this new serotype of *E. coli* by injecting rabbits[32] with a suspension of the first strain isolated from a fatal case (C. H.). This antiserum had an agglutinin titer for the thermostable somatic O antigen of 12,800 and reacted with the thermolabile envelope B antigen by slide agglutination at a dilution of 200. Homologous types of *E. coli* isolated from 43 patients were agglutinated to the maximum O titer, and B agglutination occurred immediately in dilution 1:10. Ewing subsequently confirmed the identification of these 43 strains as *E. coli* O127:B8. Strains of *E. coli* O111:B4 and O55:B5 and strains of *E. coli* commonly found in stool cultures were not agglutinated by *E. coli* O127:B8 antiserum.

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* We are indebted to C. W. Christensen of the Difco Laboratories, Detroit, Mich., for supplying us generously with this medium.

† Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga.

‡ Personal communication.

Clinical Study

The onset of diarrhea in some patients was sudden and severe, with toxemia and fever. In others the onset was gradual, with a few loose stools and illness of mild degree. In most patients, the diarrhea was intermittent, one or more days of normal stools followed by one or more days of diarrhea. Marked abdominal gaseous distention in some patients preceded the explosive onset of diarrhea. A pungent, musty, objectionable odor, not noticed around other patients with diarrhea, was present. Otherwise, the clinical picture presented by these infants resembled that of babies with diarrhea caused by *Shigella* or *Salmonella*.

Rectal swabs from a total of 158 infant patients and 82 adult personnel servicing the infant wards were cultured for *Shigella*, *Salmonella*, and specific serotypes of *E. coli*. *E. coli* O127:B8 was isolated from 44 infant patients, one showing, in addition, the presence of *E. coli* O111:B4, and another showing, in addition, the presence of *Salmonella typhimurium*. This new serotype of *E. coli* was also isolated from the rectal swab culture of one nurse who was in attendance on the infant wards and who had no history of recent diarrhea. Cultures from 13 other patients revealed the presence of *Salmonella*, *Shigella*, or *E. coli* O111:B4, and were negative for *E. coli* O127:B8. Thirty-five of the 44 patients with rectal swab cultures positive for *E. coli* O127:B8 were detected with the first culture, 6 with the second culture, and 3 with a fourth culture. Securing more than 4 cultures on any one patient did not increase the number of positive results.

Age distribution. Forty-six per cent of the infants whose rectal swab cultures were positive for *E. coli* O127:B8 and 33 per cent of those with negative cultures were in the first month of life. In the second to sixth months of age, the percentages of positive and negative cultures were comparable, 36 and 37 per cent respectively.

Incidence of diarrhea. One hundred and thirteen of the 145 infants studied had diarrhea at one time or another during their hospitalization. Forty-three of the 44 patients whose rectal swabs were positive had diarrhea, 11 of these were admitted with diarrhea, and 32 developed diarrhea in the hospital. Seventy of the 101 patients with negative cultures had diarrhea, 37 were admitted with diarrhea, and 33 developed diarrhea after admission. Of the patients who developed diarrhea after admission to the hospital, 23 (72 per cent) of the 32 patients with positive cultures and 28 (85 per cent) of the 33 patients with negative cultures developed the diarrhea during the first week.

Thirty-one of the 43 patients with positive cultures and 31 of the 70 patients with negative cultures had an intermittent type of diarrhea, with an average duration of 22 days, 9 of the patients with positive cultures and 33 of the patients with negative cultures had a continuous type of diarrhea.

Chemotherapy. Our choice of neomycin for use in this outbreak was influenced by the recent report by Wheeler and Wainerman[27] of their success with neomycin in controlling and eradicating an epidemic of diarrhea among infants due to *E. coli* O111:B4. Over the four-month period of this study, the oral dosage of neomycin for patients with positive cultures was increased from an average daily dose of 29 to 55 mg./kg./day, and for the patients without positive cultures the dosage was increased from 40 to 48 mg./kg./day. The daily dose of neomycin was divided into six equal doses and administered every four hours. Thirty-six of our 44 patients who had diarrhea and positive rectal swab cultures were treated with neomycin, and 43 of 70 patients who had diarrhea and negative rectal swab cultures were also treated with neomycin. With a continuing high incidence of diarrhea, neomycin, 40 to 50 mg./kg./day for 10 days, was administered to all patients on the two infants' wards, regardless of clinical condition. This was followed by a rapidly decreasing incidence of diarrhea and decreasing detection of *E. coli* O127:B8. No evidence of toxicity attributable to neomycin was observed. Although neomycin was of clinical value therapeutically, it was inadequte bacteriologically in the dosage used, since 12 of 22 infants continued to show the presence of *E. coli* O127:B8 after discontinuing neomycin.

Mortality. There were 4 deaths among the 44 infants whose rectal swab cultures were positive for *E. coli* O127:B8. Necropsy studies revealed intestinal pathology in 3 patients, which no doubt contributed to their deaths. In the fourth patient, a congenital heart condition was the most likely cause of death. The death of only one of the 10 patients in whom *E. coli* O127:B8 was not found may have been related to a severe diarrhea.

Sensitivity tests with six antibiotics and sodium sulfadiazine. Forty-two strains of *E. coli* O127:B8 were tested by the test tube serial dilution method for their sensitivity to dihydrostreptomycin,* polymyxin B sulfate,* chloramphenicol,* Terramycin,* neomycin sulfate,† and Achromycin.‡ The medium used was Bacto Brain Heart Infusion, and the inoculum of bacteria per ml. was approximately 250. The tubes were incubated at 37°C. and, after 20 hours, tubes that failed to show visible growth were subcultured by transferring one loopful, 0.01 ml., to tubes containing 2.0 ml. of Bacto Brain Heart Infusion and incubating at 37°C. The data in FIGURE 1 and FIGURE 2 supply information regarding the concentrations of antibiotics showing complete inhibition of growth of the 42 strains at 20 hours, as well as information regarding the concentrations that were bactericidal at that time. The antibiotics listed in the order of their decreasing effectiveness in inhibiting visible growth at 20 hours are as follows: polymyxin, Achromycin, chloramphenicol, Terramycin, and neomycin sulfate. The order of decreasing bactericidal effectiveness was: polymyxin, neomycin sulfate, chloramphenicol, Achromycin, and Terramycin. These data indicate that polymyxin was the most effective, since smaller quantities inhibited visible growth of, and proved to be bactericidal for, a larger number of strains. Neomycin sulfate was second most effective as a bactericidal agent. All strains of *E. coli* O127:B8 were resistant to 100 μg./ml. of dihydrostreptomycin.

Preliminary tests for sensitivity to sodium sulfadiazine, using the test tube method, revealed that three strains of *E. coli* O127:B8 were resistant to 2.0 mg. per ml. Using Bacto-Mueller-Hinton agar medium and Bacto sensitivity disks, 23 strains of *E. coli* O127:B8 were found to be resistant to 250 μg. of sodium sulfadiazine, the maximum quantity tested.

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† Kindly supplied by Harold R. Reames of The Upjohn Company, Kalamazoo, Mich.
‡ Courtesy of Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

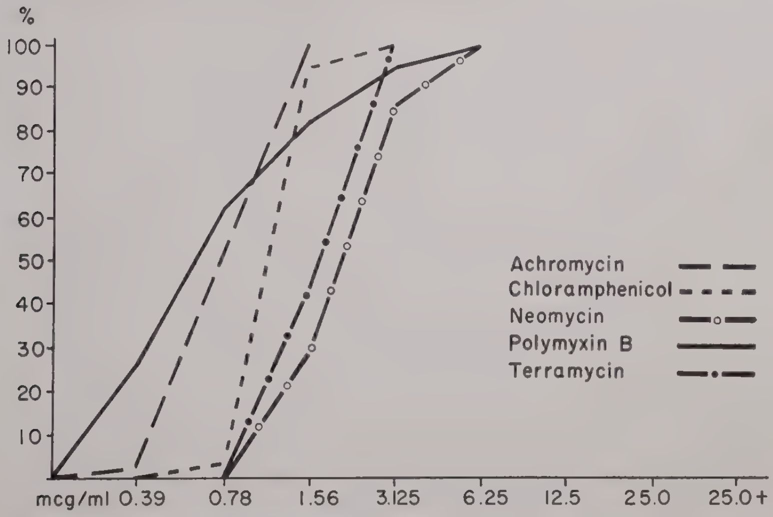

FIGURE 1. Results of antibiotic sensitivity tests on 42 strains of *E. coli* O127:B8—*inhibition* of growth at 20 hours.

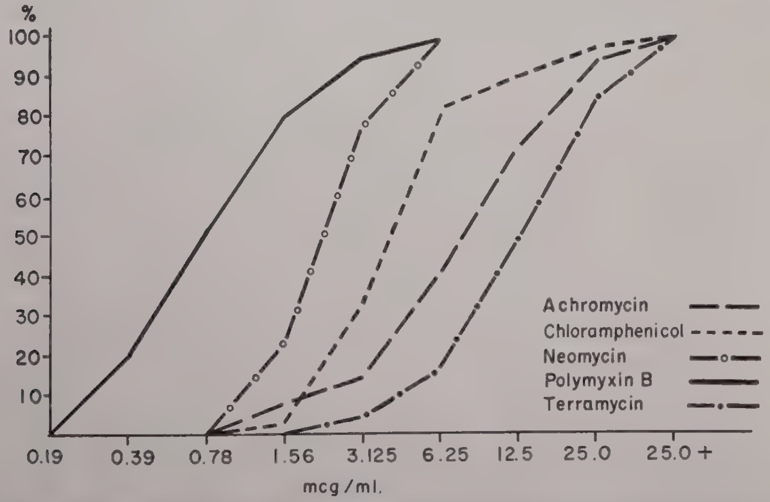

FIGURE 2. Results of antibiotic sensitivity tests on 42 strains of *E. coli* O127:B8—*bactericidal* results at 20 hours.

Agglutination studies with sera from patients and personnel. Bacterial agglutination studies with a standardized suspension of *E. coli* O127:B8 and serial dilutions of 57 sera from 24 patients and 16 personnel failed to show an agglutinin titer of 1:20 or higher. Twenty-two of these sera were then tested for antibody content by a hemagglutination test. A similar method has been used for the demonstration of antibodies against the tubercle bacilli by Middlebrook and Dubos;[38] *Hemophilus influenzae* type *b* by Warburton, Keogh, and Williams;[39] streptococci by Kirby;[40] *Pasteurella pestis* by Amies;[41] *E. coli* O55:B5 and O111:B4 by Neter, Bertram, and Arbesman,[42] and *E. coli* O55:B5 by Neter, Zalewski, and Ferguson.[43] We used a purified polysaccharide antigen prepared from a strain of *E. coli* O127:B8 according to the method of Webster, Sagin, Landy, and Johnson.[44] Patients' sera were first adsorbed with one-day-old chick red blood cells in order to remove any normal antibodies for chick cells. These cells were then discarded. The purified antigen was adsorbed onto other washed fresh one-day-old chick cells. These antigen-treated cells were added to twofold dilutions of patients' sera in small depressions or cups in plastic trays, incubated at 37°C. for two hours and kept at 4°C. for 12 to 18 hours before reading. The results (TABLE 1) are expressed as the reciprocal of the dilution in which there was at least a two-plus agglutination. It is observed that the range of the titers is from less than 4 to 1024, indicating that with a more sensitive type of agglutination technique it is possible to demonstrate antibody responses in some of these young infants. In addition, hemagglutinin titers, not shown in TABLE 1, were determined on two sera obtained from an infant and a nurse who had no recent history of diarrhea. Serum from the infant patient (J. N.), two and one-half months of age, showed a titer of 1:8. Serum from a nurse (M. K.) in attendance on the infant wards during the epidemic, and whose rectal swab cultures were negative for *E. coli* O127:B8, showed a hemagglutinin titer of 1:512.

Table 1

E. coli O127:B8 Hemagglutinin Response to Infection with *E. coli* O127:B8

	Diarrhea		Reciprocal of hemagglutinin titer
	Age at onset Days	Serum after onset Days	
R. B.	2	11, 19	64, 16
R. J.	2	77	32
R. A.	5	13, 27, 36, 73	<4, <4, <4, 8
C. C.	9	7	<4
R. K.	11	13	<4
K. D.	22	63	256
W. H.	70	71	512
C. L.	86	28	64
J. N.	103	5	32
R. H.	142	15, 20, 22, 86	512, 256, 128, 32
J. F.	396	22	1024
J. B.	Nurse	No diarrhea	8, 8

Incidence of Enteropathogenic E. coli, Shigella, *and* Salmonella *During a 19-Month Study*

Since the subsidence of our *E. coli* O127:B8 epidemic in the Children's Hospital in March 1954, we have made three changes in our bacteriological studies in relation to intestinal pathogens. First, in order to protect infants on our infants' wards, we have required admission rectal swab cultures for *Salmonella*, *Shigella*, and nine serotypes of *E. coli*. Second, all rectal swab cultures for intestinal pathogens include search for the nine serotypes of *E. coli*, in addition to *Salmonella* and *Shigella*. Third, a polyvalent *E. coli* antiserum prepared in our research laboratory is used for preliminary screening of colonies or swipes from growth on media in Petri dishes for B agglutination. This polyvalent antiserum agglutinates each of nine serotypes of *E. coli* considered by Ewing to be associated with epidemic diarrhea of infants. These serotypes are: O26:B6, O55:B5, O86:B7, O111:B4, O119:B14, O125:B15, O126:B16, O127:B8, and O128:B12. If B agglutination occurs with the polyvalent antiserum, the culture is tested for B agglutination with each of the nine *E. coli* antisera. If B agglutination occurs with one of the nine antisera, an O antigen is prepared and its agglutinability determined.

During this 19-month study, March 1, 1954 to October 1, 1955, rectal swabs from 2169 infants and young children were cultured. *Salmonella* were isolated from 66 patients, *Shigella* from 77 patients, and one of nine serotypes of *E. coli* associated with diarrhea of infants from each of 125 patients. The serotypes of *Salmonella* and the number of patients from whom each was isolated are presented in TABLE 2, where it is seen that *Salmonella oranienburg* and *Salmonella typhimurium* were detected most frequently. Ninety-four per cent of these patients had diarrhea.

TABLE 3 shows that *Shigella sonnei* was isolated more frequently than were members of the *Shigella flexneri* group. Diarrhea was present in 95 per cent of the patients from whose rectal swab cultures *Shigella* had been isolated.

The serotypes of *E. coli* isolated from 125 infants are presented in TABLE 4, where it is observed that *E. coli* O55:B5 was isolated most frequently from 46 infants, while *E. coli* O127:B8 was isolated from only 10 infants in the 19 months following subsidence of our epidemic. Diarrhea was present in 92 per cent of the infants from whom one of the nine serotypes of *E. coli* had been

TABLE 2

SALMONELLA SEROTYPES ISOLATED FROM 66 INFANTS AND YOUNG CHILDREN DURING A PERIOD OF 19 MONTHS—MARCH 1, 1954 TO OCTOBER 1, 1955

S. oranienburg	19 patients
S. typhimurium	10 patients
S. montevideo	7 patients
S. enteritidis	6 patients
S. newport	6 patients
S. schwarzengrund	5 patients
S. stanley	3 patients
Other serotypes	10 patients

TABLE 3

SHIGELLA SEROTYPES ISOLATED FROM 77 INFANTS AND YOUNG CHILDREN DURING A PERIOD OF 19 MONTHS—MARCH 1, 1954 TO OCTOBER 1, 1955

Shigella flexneri 1	4 patients
Shigella flexneri 2	16 patients
Shigella flexneri 3	4 patients
Shigella flexneri 4	3 patients
Shigella flexneri 5	2 patients
Shigella sonnei	48 patients

TABLE 4

ESCHERICHIA COLI SEROTYPES* ISOLATED FROM 125 INFANTS BETWEEN MARCH 1, 1954, AND OCTOBER 1, 1955

E. coli O55:B5	46 patients
E. coli O26:B6	17 patients
E. coli O111:B4	15 patients
E. coli O119:B14	11 patients
E. coli O127:B8	10 patients
E. coli O126:B16	10 patients
E. coli O125:B15	9 patients
E. coli O128:B12	4 patients
E. coli O86:B7	3 patients

* Nine serotypes associated with diarrhea of infants.

isolated. Neomycin was administered orally in a dosage of 150 mg./kg./day for two days, followed by a dosage of 100 mg./kg./day for the following eight days. In these dosages, neomycin was of value not only therapeutically but also bacteriologically. There were only three deaths among the 125 patients, and *E. coli* O55:B5 had been isolated from each before death. Also, only three convalescent carriers were detected with rectal swab cultures on 44 patients after discontinuing neomycin therapy.

Summary

During an outbreak of epidemic diarrhea in the Children's Hospital, Cincinnati, Ohio, a new serotype of *Escherichia coli: E. coli* O127:B8, was isolated from 44 of 145 infants and from 1 nurse among 82 adult personnel in attendance.

Severe epidemic diarrhea associated with the presence of *E. coli* O127:B8 was characterized by the sudden development of extreme abdominal distention among some of the infants; explosive onset of diarrhea, and the presence of a pungent, musty, objectionable odor not noticed around other patients with diarrhea.

E. coli O127:B8 was isolated more frequently while the patients were having diarrhea.

Neomycin was used orally for the specific treatment of patients with diarrhea. The average dose was 40 mg./kg./day for the patients with positive cultures and 46 mg./kg./day for those with negative cultures.

Neomycin was of clinical value therapeutically but was inadequate bacteriologically in the dosage used, since 12 of 22 patients continued to show the presence of *E. coli* O127:B8 after discontinuing neomycin.

The administration of neomycin, 40 to 50 mg./kg./day, to every infant on the two wards, regardless of their clinical condition, was followed by a decreasing incidence of diarrhea and decreasing detection of *E. coli* O127:B8.

Four deaths occurred among the 44 infants whose rectal swab cultures were positive for *E. coli* O127:B8. Necropsy studies revealed hemorrhagic enteritis in 3 infants and a congenital heart condition in the fourth infant. Death of only 1 of the 10 fatal cases from whom *E. coli* O127:B8 had not been isolated may very likely be attributed to severe diarrhea.

Sera from patients and personnel failed to show the presence of bacterial agglutinins for *E. coli* O127:B8. Hemagglutination studies, however, revealed a range of titers from less than 4 to 1024.

In vitro sensitivity tests showed that the order of decreasing bactericidal effectiveness of five antibiotics for *E. coli* O127:B8 was polymyxin, neomycin, chloramphenicol, Achromycin, and Terramycin. All strains were resistant to dihydrostreptomycin and sodium sulfadiazine.

During the 19 months since the subsidence of our epidemic of diarrhea in which *E. coli* O127:B8 was detected, rectal swabs have been obtained from 2169 infants and young children. Cultures of these swabs resulted in the isolation of *Salmonella* from 66 patients, *Shigella* from 77 patients, and 1 of 9 serotypes of *E. coli* associated with diarrhea of infants from each of 125 patients.

Neomycin, administered orally to patients whose rectal swab cultures were positive for 1 of the 9 serotypes of *E. coli*, was of value therapeutically and bacteriologically.

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INFANTILE DIARRHEA DUE TO *ESCHERICHIA COLI*[*]

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The significance of certain serotypes of *Escherichia coli* as etiologic agents in infantile diarrhea has come principally from studies of epidemics and experimental infections in adult human volunteers. The pioneering investigations of the British and the equally outstanding contributions of Neter, of Ferguson, and of Wheeler in this country set the stage for the developments that firmly established *E. coli* as a cause of epidemic diarrhea in infants.

In Detroit, Mich., we have had an opportunity to study two outbreaks that have provided us with unusual information and enabled us to investigate new aspects of epidemic diarrhea of the newborn. This report will be divided into three parts: first, a review of the epidemiology and bacteriology of the epidemics[1, 2] that provides ample evidence for the causative agents; second, an examination of the immunology of the premature infants involved in one of the epidemics[3] and comparative observations on a number of older infants; and, third, observations on the incidence of recognized serotypes among a large group of sporadic cases.

Part 1

In two extensive institutional epidemics of diarrhea among premature infants, the causative agents were shown to be *E. coli* O111:B4 and O127:B8, respectively.[1, 2] The etiologic role of these microorganisms was evident from their presence in virtually pure culture during the disease and by the almost simultaneous disappearance of both symptoms and organisms under effective antibiotic therapy. In both epidemics it was observed that after therapy was discontinued the infants almost invariably excreted the pathogens again, often in pure culture, and often in the absence of renewed diarrheal symptoms. In the second epidemic (FIGURE 1), these asymptomatic carriers were responsible for four connected epidemic waves in the nursery. Although, during most of the epidemic, the etiologic agent was not known, stool specimens and cultures were systematically saved from each infant until the identity of the causative organism could be established as *E. coli* O127:B8. A retroactive study of the epidemic by serotyping then revealed the bacteriologic basis for its cyclic pattern. Each outbreak was preceded by the reappearance of large numbers of

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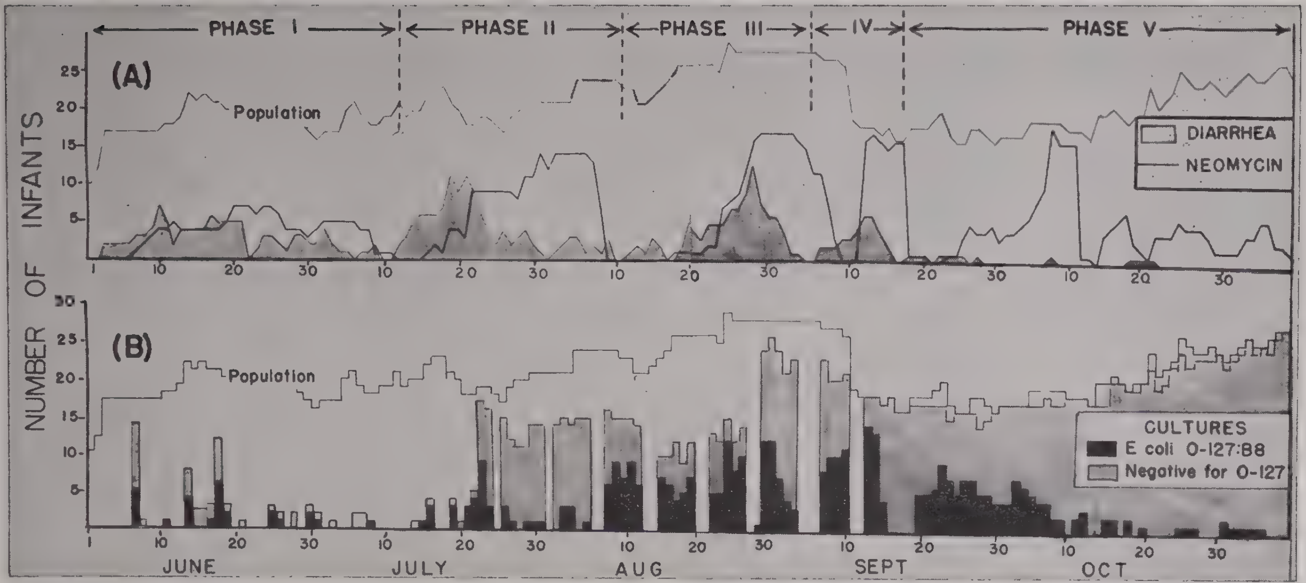

FIGURE 1. Relation of *E. coli* O127:B8 to an epidemic of diarrhea in a premature nursery. A, daily incidence of diarrhea and the effect of antibiotic therapy on the course of the epidemic. B, incidence of stool cultures positive and negative for *E. coli* 0127:B8 in relation to diarrheal disease and treatment. Reproduced by permission of the *American Journal of Diseases of Children.*

E. coli O127:B8 in the stools of the convalescent infants. Each wave subsided when neomycin was administered to the sick infants, only to result in a resurgence of the pathogenic organisms in the stools as soon as the antibiotic was discontinued. Control of the epidemic was achieved only when all the infants in the unit, whether they had diarrhea or not, were simultaneously placed on neomycin therapy and continuously treated until the daily bacteriologic examination of the stools revealed that the pathogens had been eliminated from the nursery.

Part 2

It was of immediate interest to learn whether the infants involved in the *E. coli* O127 epidemic developed an immunologic response to their infections. The evanescent character of the antibody response in newborn and young infants, in whom diarrhea due to enteropathogenic *E. coli* occurs almost exclusively, has been ascribed to the inadequacy of conventional bacterial agglutination techniques to detect circulating antibodies in this age group. Consequently, a hemagglutination test was developed for the recognition and assay of antibodies against enteropathogenic strains of *E. coli* which was used to study the antibody response of these infants.[3] The hemagglutination procedure, based on techniques developed by Neter *et al.*[4] and by Wheeler[5] and his associates, involved the exposure of trypsinized human erythrocytes to filtrates of tryptose phosphate broth cultures of enteropathogenic *E. coli* strains and resulted in adsorption of bacterial antigenic material on the red cells within four minutes. Hemagglutination occurred as a result of exposure of the sensitized cells to specific antisera.* After this test had proved satisfactory with experimental rabbit antisera, the sensitivity of the hemagglutination test and the specificity of hemagglutinating antibody in human sera was established in two adult volunteers who were fed large numbers of *E. coli* O127:B8.[3, 6] Following ingestion of the organisms and after a brief but severe diarrhea, these volunteers developed both hemagglutinating and bacterial-agglutinating antibodies. The hemagglutinins not only appeared earlier and were of higher titer than the bacterial agglutinins, but both individuals also manifested low prefeeding hemagglutinin titers in the absence of demonstrable bacterial agglutinins. Cross-absorption tests with a heterologous enteropathogenic strain (*E. coli* O111:B4) revealed that both the prefeeding and postfeeding hemagglutinins were specific.

The presence of traces of specific hemagglutinating antibody in the volunteers before ingestion of *E. coli* O127:B8 suggested that such antibody may be commonly found in normal adults and consequently might be passively transferred from mother to fetus. That this was the case was determined from the study of the sera of a number of healthy newborn infants and their mothers.[3] Of ten mothers, seven had hemagglutinins for *E. coli* O127-sensitized erythrocytes, while in the cord blood of only two of their infants (from mothers with highest titers) was antibody detectable, and this eventually disappeared. Additional information on the passive transfer of antibody to *E. coli* O127:B8 was obtained from the study of cord sera of 45 normal infants, 27 per cent of whom had hemagglutinating antibodies ranging in titer from 1:10 to 1:80.

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* The relationship of the adsorbed antigen in this system to O and B antigens of the intact bacterium has not yet been established.

With these observations in mind, a more adequate interpretation could be made of the presence of hemagglutinating antibodies in infants involved in the *E. coli* O127:B8 epidemic mentioned above.[3] It is important to remember that these patients were premature infants and that somewhat different antibody patterns might be found in full-term newborn or older infants. Of 40

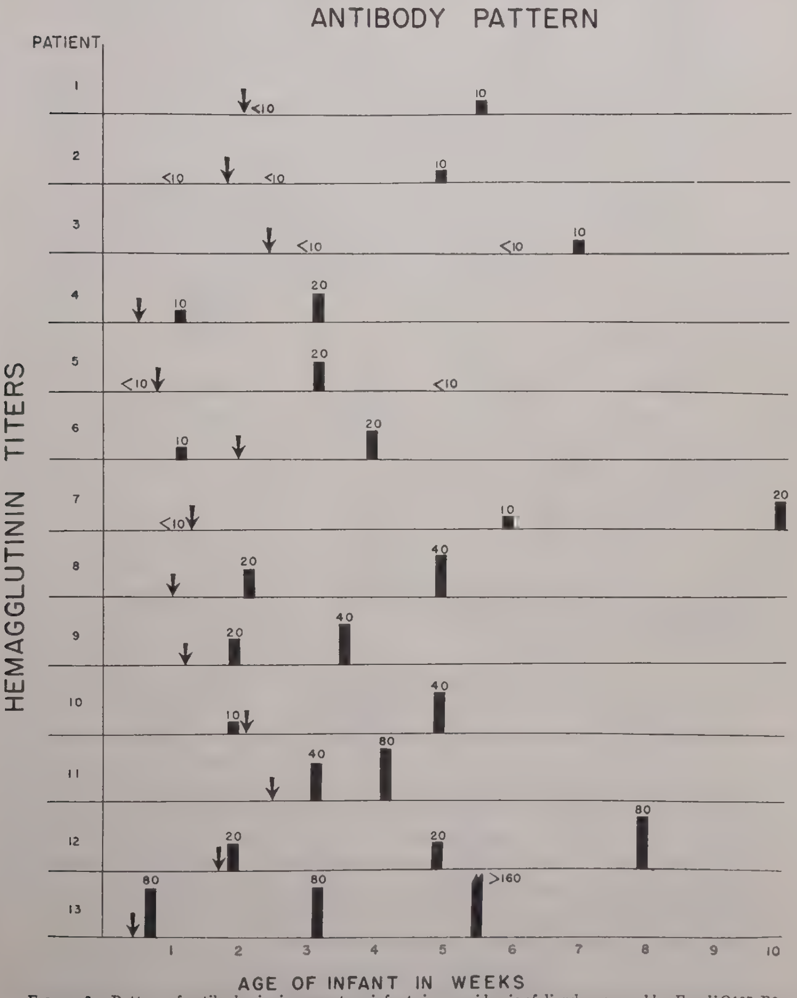

FIGURE 2. Pattern of antibody rise in premature infants in an epidemic of diarrhea caused by *E. coli* O127:B8. Arrow denotes the onset of disease in each individual. Reproduced by permission of *The Journal of Immunology*.

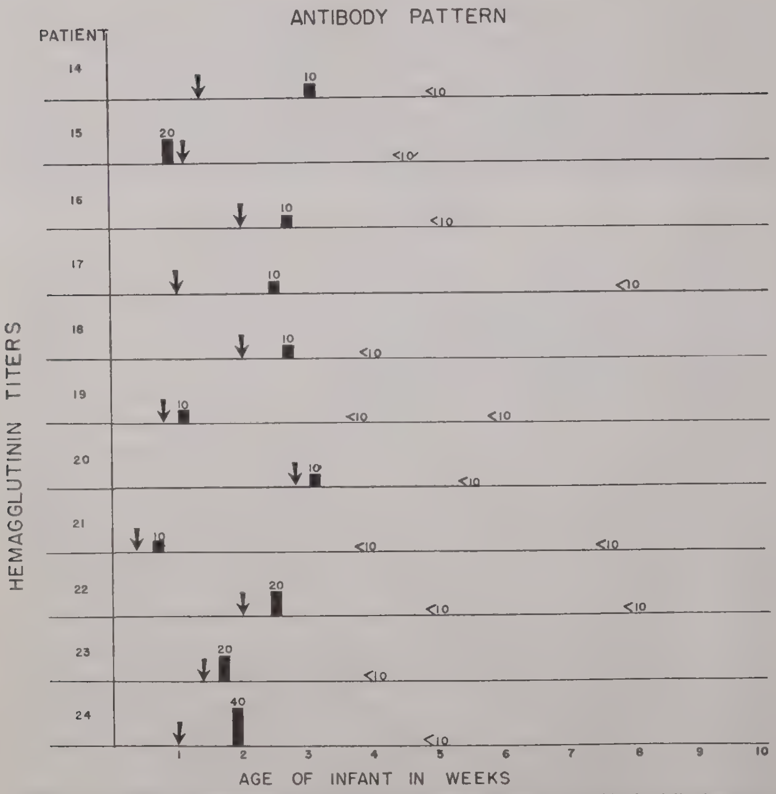

FIGURE 3. Decline in transplacental antibody titers in premature infants in an epidemic of diarrhea caused by *E. coli* O127:B8. Arrow denotes the onset of disease in each individual. Reproduced by permission of *The Journal of Immunology*.

infants from whom adequate blood specimens could be obtained, 13 manifested a rise in antibody titer (FIGURE 2). Approximately half of the 13 had some antibody before or at the onset of disease which, because of the ages of the infants, was presumed to be of maternal origin. Of the remaining 27 infants, 11 showed a decrease in transplacental antibody with no increase in the titer following infection (FIGURE 3); 13 infants did not have detectable antibody at any time; and 3 infants had ambiguous antibody patterns.

It is of interest to examine the bacteriologic, clinical, and thereapeutic findings on a few of these infants with respect to their antibody patterns. FIGURE 4 diagrammatically illustrates 4 patients who exemplify the 40 on whom immunologic studies have been previously reported.[3]

In the first patient, the pathogen was observed in the stools in practically pure culture at the onset of and during the disease. When neomycin was administered, the stools became sterile and diarrhea ceased. After the antibiotic

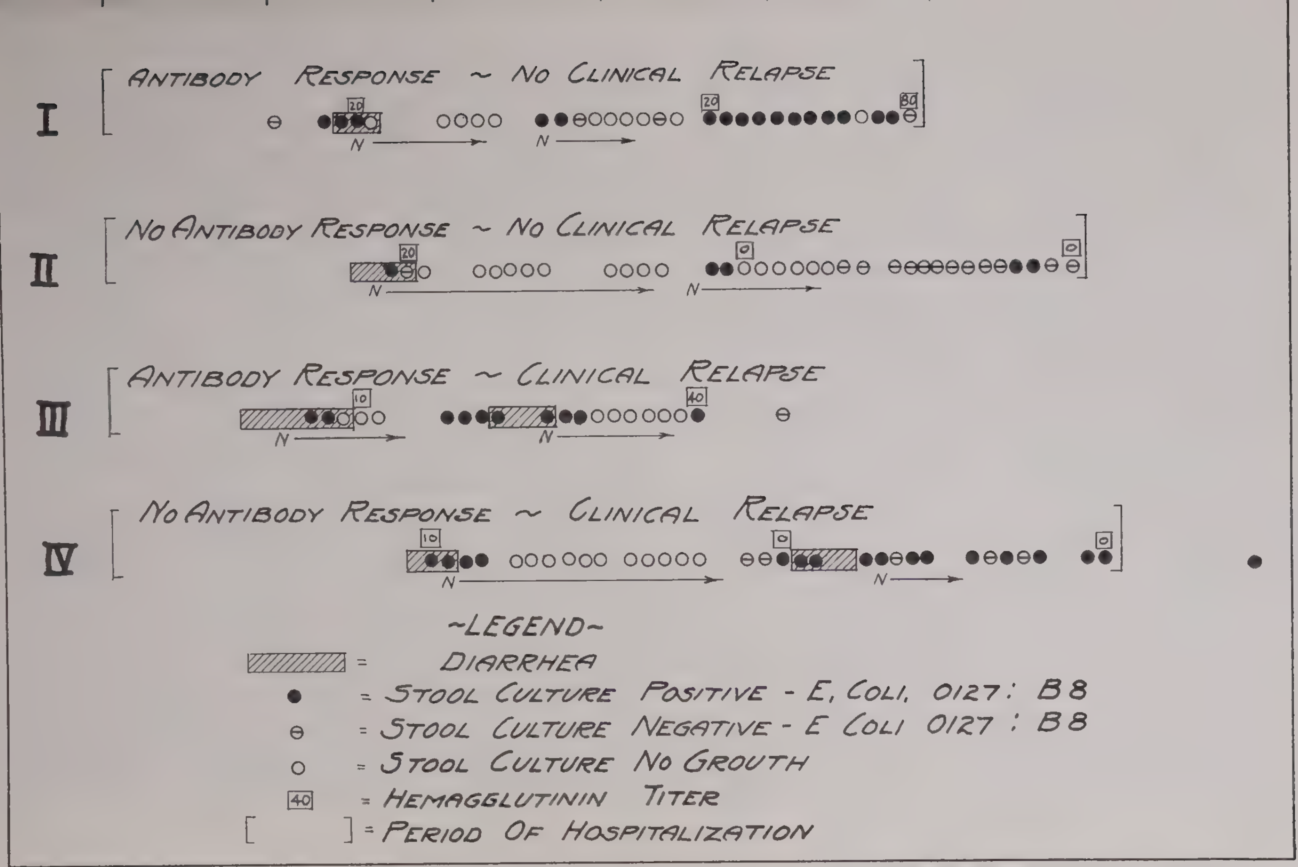

FIGURE 4. Bacteriologic, immunologic, and clinical studies on selected cases from an epidemic of diarrhea due to *E. coli* O127:B8. *N* indicates administration of neomycin.

was discontinued, the infant again excreted *E. coli* O127, but the prompt readministration of neomycin again eliminated the pathogen. A third bacteriologic relapse occurred, however, when treatment was again discontinued and large numbers of *E. coli* O127 appeared in the absence of renewed clinical symptoms. The hemagglutinin titer of this infant was 1:20 at the onset of disease and, by the time he was discharged 37 days later, the antibody titer had increased to 1:80. The second infant had a somewhat similar clinical course although, after therapy was stopped, the reappearance of pathogens was limited to a shorter period. In this patient a trace of antibody was observed at the onset of diarrhea but no antibody could be detected when he was discharged 36 days later.

The next two infants exhibited both bacteriologic and clinical relapses; one in spite of an antibody rise, the other in the absence of detectable antibody.

These examples not only illustrate the bacteriologic and clinical patterns characteristic of infantile diarrhea due to *E. coli* but indicate that the presence of small amounts of antibody, whether maternal or not, established no protection against the clinical disease in very young infants.

From these data it is difficult to ascribe the variation in antibody response to any one factor. The nature of the antigenic stimulus, individual variations in ability to produce the antibody (especially in this age group), and the possibility that antibiotic therapy reduced the antigenic stimulus, all may have been influential.

It is interesting to compare the antibody patterns of the premature infants in the epidemic to those of older infants who had been infected with enteropathogenic *E. coli* and had developed diarrhea. The latter, illustrated in TABLE 1, represent preliminary immunologic studies on infants under one year of age admitted to the Children's Hospital of Michigan for treatment. The selection of these particular patients for this presentation was based on several considerations; namely, that more than one blood specimen was available, that the serotypes isolated from the stools (*E. coli* O111:B4 and O127:B8) are accepted causative agents of infantile diarrhea, and that the bacteriologic, clinical, and therapeutic findings in the individual infants conformed to patterns established in epidemic studies. The data shown in TABLE 1 indicate a variability in antibody response similar to that observed in the premature infants involved in the epidemic. Of the 14 infants listed in TABLE 1, 6 showed an antibody rise to the infecting strain which was made evident by a 4- to 8-fold difference between the acute and convalescent hemagglutinin titers. In addition, 4 had high initial titers, whereas their convalescent titers remained approximately the same. Of the remaining 4 patients, who either exhibited no antibody or low titers, 2 were treated with neomycin within 24 hours after the onset of diarrhea and continuously thereafter, so that after one positive stool culture, all subsequent cultures (performed daily) were negative. Hence, it is conceivable that their antigenic stimulus was cut short. On the whole, there appeared to be no correlation between the ages of these infants and their ability to develop antibody.

Wherever possible, serum specimens from these infants were also tested against three heterologous enteropathogenic *E. coli* antigens, as seen in TABLE 1. With few exceptions, the hemagglutinin titers were generally low

TABLE 1

ANTIBODY PATTERNS OF SPORADIC CASES OF INFANTILE DIARRHEA DUE TO ENTEROPATHOGENIC *E. COLI*

Patient	Serotypes in stools	Age at onset (months)	Interval between onset and serum specimen (days)	Hemagglutinin titers* against			
				O127	O111	O55	O26
1	O127	3	6	20	40	0	0
			14	20	20	—	—
			20	80	40	—	—
2	O127	1½	1	40	320	0	0
			10	160	160	0	0
3	O127	2	34	80	10	—	—
			44	160	0	0	0
4	O127	3	5	40	40	—	—
			13	20	10	—	—
5	O127	6	1	0	0	—	—
			7	20	0	0	0
			28	0	0	0	0
6	O127	3	2	0	10	80	10
			16	0	10	10	0
7	O127	9	3	10	0	20	20
			14	0	0	20	—
8	O111	½	21	40	20	10	10
			35	40	160	0	10
9	O111	3	16	10	20	10	20
			23	20	160	10	0
10	O111	6	5	80	20	—	—
			16	40	80	20	20
11	O111	1½	4	10	160	0	40
			16	20	80	0	40
12	O111	6	4	40	160	0	10
			13	10	160	0	10
13	O111	3	14	0	80	0	10
			22	0	40	0	10

* Reciprocal of the dilution.

and there was no pattern of antibody response. Indeed, the presence of antibody to heterologous antigens in this age group corresponded to the observations of Neter and his co-workers[7] who, in a random sample, have demonstrated that with age there is a progressively increasing percentage of sera containing *E. coli* hemagglutinins in relatively low titer.

These data invite some speculation on differences in the manifestation of the infection in infants and in adults. The fact that adults have little or no protective antibody[3, 6-8] and yet appear to be normally resistant to *E. coli* diarrhea would lead one to suspect that resistance is due primarily to factors other than humoral antibodies. One such factor may be that the ecology of the adult intestinal flora normally prevents an enteropathogenic *E. coli* from becoming dominant. This notion would concur with the observation that if the normal ecology of the gut is upset by massive feeding of the pathogen, diarrhea ensues.[6, 8] The diarrhea could conceivably be due to a concentration of otherwise mild endotoxin, liberated from the excessive numbers of organisms ingested. Under such circumstances, the adult rapidly produces antibody which reaches protective levels,[3, 6] and a second line of defense, so to speak, becomes manifest. The ecology of the infant's intestinal flora, on the other hand, may be such that, in the natural infection, the pathogen becomes the predominant if not the sole coliform organism. Diarrhea follows, and the inadequate immunologic response of the infant results in the characteristic clinical picture of long periods of diarrhea and frequent relapses. In other words, the infant would appear to have a poorly developed first and second line of defense.

Part 3

A consideration of endemic diarrhea in the general infant population is limited to the picture reflected by those cases admitted to the hospital for treatment. The problem is different from that of institutional epidemics where one is dealing with a single causative agent; the patients exhibit similar patterns, cross-infections can be followed, and the effect of therapy can be followed not only on individuals but on the epidemic as a whole. Conversely, in sporadic cases there may be a number of different causative agents resulting in various clinical patterns, and preliminary and follow-up information may be difficult to obtain. Over a nine-month period, data were collected on all infants admitted to the Children's Hospital of Michigan for treatment of acute diarrheal disease. Stool cultures were obtained on admission and daily thereafter. Rectal swabs were plated directly on blood agar for identification of eight enteropathogenic *E. coli* serotypes, on MacConkey's, on SS, and on tetrathionate media for preliminary isolation of enteric pathogens of the *Salmonella* and *Shigella* groups. In TABLE 2, 460 patients are separated into three principal groups: those that were associated with enteropathogenic *E. coli*, those from whom *Salmonella* and *Shigella* were isolated, and those where the etiology is as yet unknown.

Recognized serotypes of *E. coli* were isolated from 106 cases. Extensive studies of O127, O111, and O55 epidemics have established these pathogens as causative agents of diarrhea, and it is apparent that well over half of the pa-

TABLE 2
CLASSIFICATION OF SPORADIC CASES OF INFANTILE DIARRHEA
ADMITTED TO THE CHILDREN'S HOSPITAL OF MICHIGAN
OCTOBER 1, 1954, TO JUNE 1, 1955

Categories	Total number	O26	O55	O86	O111	O119	O125	O126	O127
Associated with enteropathogenic *E. coli*	106	3	9	1	7	4	4	25	53
E. Coli present but not belonging to 8 serotypes	287	Possible etiology: Unknown *E. coli* enteropathogen? Other unknown bacterial enteric pathogens? Missed enteric pathogens? Parenteral diarrheas? Feeding problem? Allergies? Viruses?							
Undetermined	52								
Salmonella	10								
Shigella	4								

tients in this group can be attributed to these three serotypes. The other serotypes were found in patients with similar clinical, bacteriologic, and therapeutic patterns, which would lead one to conclude that they, also, are causative agents.

Salmonella and *Shigella* organisms were isolated from only 14 patients, a figure that may not give the true incidence since a majority of patients had had chemotherapy effective against these agents before admission to the hospital.

At this time the causes of diarrhea in the remaining 339 cases are obscure. Of these, 287 had *E. coli* in their stools as the predominant organism but not of the recognized serotypes. It may be significant that these organisms almost invariably agglutinated in acriflavine, whereas the eight recognized serotypes did not. This phenomenon is discussed by Wheeler elsewhere in this monograph. Whether any of the etiologic possibilities listed in TABLE 2 are responsible for the diarrheas grouped in this category remains to be determined.

Summary

Infantile diarrhea due to enteropathogenic *E. coli* has been discussed from the standpoint of our experience with epidemic and sporadic cases. The epidemiology, bacteriology, and clinical aspects of the epidemics provided ample evidence of the etiologic agents *E. coli* O111:B4 and O127:B8. The role of the asymptomatic carriers in relation to the propagation of institutional epidemics was discussed. The occurrence of an antibody response in the infants involved in one of the epidemics and in sporadic cases added to the etiologic significance of these microorganisms. By means of a hemagglutination test it was shown that passive antibody against enteropathogenic serotypes may exist in the normal newborn, and that a variable antibody response took place in the infants as a result of the infection with these organisms.

During a survey of a large number of sporadic cases it was found that 23 per cent were due to enteropathogenic *E. coli*, while the etiologies of the remainder are yet to be established.

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OBSERVATIONS ON ENTEROPATHOGENIC *ESCHERICHIA COLI*

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Introduction

The mechanisms of pathogenesis of many disease-producing microorganisms remain as yet incompletely understood. This is the case with enteropathogenic *Escherichia coli*. Although colon bacilli had long been recognized as possessing a conditional pathogenicity, it was not until the observations of Kauffmann,[1] Knipschildt,[2] Ewertsen,[3] Vahlne,[4] and Sjostedt[5] that certain serological types were recognized as possessing a particular pathogenicity for man and animals. With the recognition of the etiologic role of enteropathogenic *E. coli* serotypes in diarrhea of the newborn, it appeared desirable to investigate this pathogenic action on the part of coliform bacilli that were culturally indistinguishable from the benign flora found in the healthy infant.

A distinctive aspect of neonatal diarrhea due to enteropathogenic *E. coli* is the extreme toxic state that frequently develops, with acute cases displaying recurrent episodes of a shocklike syndrome. Furthermore, in seriously ill infants and in fatal cases, a hemorrhagic diathesis has often been noted. The toxic attributes of *E. coli* have been referred to the production of a distinctive component designated variously as "somatic antigen," "endotoxin," "pyrogen," and "tumor hemorrhagic agent."[6] Kauffmann,[7] however, noted that greater toxicity of *E. coli* for mice is observed when thermolabile L antigens are present than is the case with strains lacking this component. Sjostedt and Vahlne also showed that the toxicity of *E. coli* is much enhanced by the presence of K antigen. Hence it is possible that the toxicity of these strains is due to factors in addition to the endotoxin. The assessment of pathogenicity is hampered in the case of *E. coli* strains by the fact that true infections cannot be established in small laboratory animals. The toxicity of enteropathogenic *E. coli* strains and factors contributing to hemorrhagic reactions with these organisms have been studied in our laboratory and are the subject of this report. Since the small group of reasonably well-established enteropathogenic serotypes is part of a large, culturally homogeneous bacterial species subdivided into over 130 groups on the basis of serologic distinctions, and since the great majority of these serotypes show no evidence of pathogenic potentialities, the study has been based on comparison between enteropathogenic serotypes and *E. coli* organisms not serologically related to the pathogenic forms.

Observations

Toxicity of enteropathogenic Escherichia coli. Mice, although moderately resistant to the toxic action of *E. coli*, have been extensively used to study the relative toxicity of different strains. Sjostedt[5] demonstrated the existence of marked differences in mouse toxicity among various serotypes of *E. coli*. In view of such findings, a comparison of toxicity for mice was made between en-

TABLE 1

COMPARATIVE MOUSE L.D.$_{50}$ FOR *ESCHERICHIA COLI* TYPES O111, O127, O55, O86, O26, AND O126, AND FOR NONPATHOGENIC TYPES OF *E. COLI*

Type	No. of strains	Range of L.D.$_{50}$	Mean value of L.D.$_{50}$
O111	15	1×10^7 to 1.2×10^8	3.5×10^7
O127	5	3.5×10^7 to 2.1×10^8	6.2×10^7
O55	5	2.5×10^7 to 9.1×10^7	4.1×10^7
O86	2	3.8×10^7 to 5.1×10^7	4.5×10^7
O26	3	2.1×10^7 to 7.6×10^7	4.7×10^7
O126	2	4.6×10^7 to 2.0×10^8	1.28×10^8
Nonpathogenic strains	15	3.5×10^7 to 4.7×10^8	9.1×10^7

Inoculum: 1.0 ml. of a 5-hour broth culture injected intraperitoneally.
Numbers tested: 6 to 10 animals per dilution.

teropathogenic types and nonpathogenic strains of *E. coli*. The presence of K antigens is apparently associated with increased virulence or toxicity of these strains[7] and, to ensure optimal production of these substances, the cultures were passed three to five times on thick glucose agar plates containing 0.1 per cent glucose. Subcultures of the final passage on carbohydrate agar were inoculated into trypticase soy broth. Five-hour cultures were used as inocula for mice. Injections of serial dilutions of culture were made in 1 ml. amounts intraperitoneally in 14 to 16 gm. mice of the O'Grady strain. Six to 10 mice per dilution were injected, and deaths were recorded over a 72-hour period. The results of injecting 32 enteropathogenic strains, including types O111:B4, O127:B8, O55:B5, O86:B7, O26:B6, and O126:B16, together with 15 nonpathogenic *E. coli* strains, are summarized in TABLE 1. The nonpathogenic strains mainly included organisms recently isolated from normal infants. The results of toxicity tests are presented in terms of the L.D.$_{50}$. The range of this toxicity value extended from 1×10^7 cells for the most toxic strain of O111, to 2.0×10^8 for the least toxic O126 strains. Among nonpathogenic strains the range in L.D.$_{50}$ extended from 3.5×10^7 to 4.7×10^8. In a limited series of tests, the enteropathogenic strains appeared to be significantly more toxic for mice than were nonpathogenic strains, but the gradation of results for organisms of the two groups was too uniform to indicate a significant degree of difference in toxicity between the two groups. This fact is shown more clearly in the column of compilation of mean values of L.D.$_{50}$ for all strains. The virulence levels observed were approximately 1 logarithm higher than comparable values reported by Sjostedt for certain *E. coli* strains recovered from such pathological processes as appendicitis, peritonitis, and cystitis.

Different routes of inoculation were reviewed in an attempt to uncover a more precise means of assessing toxicity. Ten groups of mice were inoculated intracerebrally, using suspensions identical with those described above. It was found that differences in virulence could be recognized in this manner, but survivors were distributed over a wider dosage range than was the case with animals receiving the intraperitoneal injections. Intravenous inoculations, both with young living cells and with sterile filtrates of cultures, were similarly tried, but results were less satisfactory than they were with the intraperitoneal inoculations. Broth filtrates from one- to six-day-old cultures were too low in mouse toxicity to offer a useful technique in titration.

The toxicity tests in mice, under the conditions employed, failed to demonstrate a significant degree of difference between enteropathogenic strains and other serotypes of *E. coli*. A limited series of observations on the toxicity of young cultures for immature rabbits indicated that significant differences may be shown between *E. coli* O111 strains and nonpathogenic serotypes when cultures were injected in this animal intravenously. Such assessment of virulence or toxicity, however, involved a prohibitive cost and, in a search for other approaches to this problem, the action of coliform organisms on tissue cultures was observed. Small numbers of bacteria from young broth cultures were planted in tubes of HeLa cell cultures that had been washed free of antibiotic. The enteropathogenic *E. coli*, particularly *E. coli* O111, were found to be lethal to an extent that made it possible to calculate, on a most-probable-number basis, that an organism would proliferate to destroy the culture within 18 hours. In certain experiments, nonpathogenic strains were found to be far less lethal, so that in some instances 1000 or more organisms were tolerated by tissue cultures. In a limited number of cases the bacterial cells actually were killed by contact with the tissue culture. This result was not consistently reproducible, and apparently variations in the tissue culture may affect the survival of bacterial cells in this environment. In no instance, however, did small inocula of enteropathogenic strains of *E. coli* fail to proliferate in tissue cultures, in contrast to the behavior of some nonpathogenic types. The possibility of assessing pathogenicity of *E. coli* strains in terms of their lethal action for tissue cultures merits further exploration.

Local tissue reactivity and enteropathogenic Escherichia coli. The pathological manifestations of fatal infections with enteropathogenic *E. coli* in infants include a marked hemorrhagic diathesis. In one epidemic outbreak studied in this laboratory,[8] the mortality rate among newborn infants was 40 per cent. In severely ill cases that survived, the major symptom was a profuse dehydrating watery diarrhea that frequently induced a state of shock. Fatal cases exhibited severe shock and bleeding from the intestine with, in three cases, coalescing skin petechiae. At autopsy, diffuse congestion and edema of the mucosa of the gastrointestinal tract was a conspicuous finding, with small areas of hemorrhage and ulceration in the small and large intestines. Grossly visible petechial hemorrhages were present in the kidneys, lungs, adrenals, and, in two instances, in the brain. In each case, microscopic examination revealed histologic evidence of diffuse intravascular clotting, with fibrin and cellular thromboses in capillaries of lung, liver, spleen, and adrenals. Perivascular infiltration with polymorphonuclear leukocytes was present. Fibrin thrombi were found occluding the lumen of the capillaries in the renal glomeruli. These lesions were morphologically identical to those seen in the kidneys of rabbits in which a generalized Shwartzman reaction had been elicited. This resemblance and further consideration of the areas of hemorrhagic necrosis of the mucosa of the small bowel and colon in fatal cases led to the thought that these lesions might represent localized reactions of the Shwartzman type, associated with the pathogenesis of diarrhea due to enteropathogenic *E. coli* types. This relationship was investigated by a study of the Shwartzman reaction in connection with enteropathogenic *E. coli*.

Organisms of the genus *Escherichia* have long been known to produce potent tissue-sensitizing substances that can stimulate the phenomenon of local tissue reactivity.[9] The relative capacity of epidemic enteropathogenic strains and of nonpathogenic strains to induce the Shwartzman reaction, however, apparently has not been recorded.

Reacting fluids were prepared, consisting chiefly of filtrates of trypticase soy-broth cultures of varying ages. As was the case with toxicity studies, the K-antigen content of cultures was maintained by serial passage on glucose agar plates. Surface antigens in recently isolated O111 strains (this group received major consideration) were conspicuous, but these components diminished quantitatively when cultures were maintained on carbohydrate-free agar. Broth-culture filtrates were prepared from cultures incubated from 1 to 7 days at 37° C., with intermittent shaking, and were sterilized by Selas filtration. Filtrates were stored at 5° C. or at −20° C. Active preparations were readily obtained from 24-hour cultures, but 6-day culture filtrates were more potent and were the principal source of test material. Whole-cell extracts were also prepared by trichloracetic acid extraction, following the method of Boivin,[10] and by treatment of whole cells with 0.05N NaOH at 100° C. for 1 hour.

Initial studies were made by inducing systemic reactions using 2 injections, at 24 hour intervals, of 0.5 ml. of *E. coli* O111:B4 filtrate. Six of 10 rabbits so treated showed positive hemorrhagic reactions in kidney, lung, thymus, and myocardium 24 hours after the evoking injection. Antigens from nonpathogenic *E. coli* strains were, under these conditions, inactive. Since it was evident that the number of tests required to obtain significant results by comparing several antigens would be large and the systemic-reaction technique permitted only one comparison per animal, subsequent comparative studies employed cutaneous reactions.

A total of 36 strains of enteropathogenic types, including O111, O55, O26, O86, O127, and O126, and 15 nonpathogenic strains, were used to prepare culture filtrates for comparison of capacity to sensitize for and to evoke the cutaneous Shwartzman reaction. The sensitizing dose consisted of 0.1 ml. of filtrate injected intradermally into the depilated abdominal skin of a rabbit. Male albino rabbits weighing from 2000 to 2500 gm. were used. Evocative doses consisted of 0.2 to 0.25 ml. of filtrate injected intravenously 20 hours after the sensitizing dose. Reactions were read at 18, 24, and 40 hours. Control tests of filtrates showed that in no instances were single injections capable of producing hemorrhagic reactions. Positive reactions included all degrees of intracutaneous hemorrhage, with or without necrosis, appearing within 24 hours after the evoking injection. Erythema, edema, or blanching reactions were not recorded as positive.

Comparison between O111 and non-O111 strains in sensitizing and evoking capacity were made with 12 strains of O111:B4 and with 18 other strains, including 3 heterologous enteropathogenic types (O86, O55, and O26) and 15 nonenteropathogenic strains, including O3, O4, O5, O6, and 11 untyped recent iso-

TABLE 2

CUTANEOUS SHWARTZMAN REACTIONS WITH CULTURE FILTRATES OF *E. COLI* O111 AND WITH NONENTEROPATHOGENIC *E. COLI*

Type	Strains	Sensitized with filtrate	Evoking antigen	Total tests	Positive reactions	Per cent positive
O111	12	O111	O111	248	211	85
O111	10	O111	Other*	96	18	19
O111	10	Other*	O111	104	38	37
—	15	Other†	Other*	242	34	14

* Includes O6, O5, O4, O3, O86, O26, and O55.
† Includes only nonenteropathogenic *E. coli* strains.

lates from normal children. These comparative reactions are summarized in TABLE 2. In 248 tests in which O111 antigens were used, 211 or 85 per cent of the prepared sites reacted to an evoking dose of culture filtrate of the same or another O111 strain. No marked difference in reactivity of different strains of O111 was noted. In most instances six tests per animal were run. When sensitization with O111 filtrates was followed by an evoking dose of heterologous filtrate, the proportion of positive reactions was only 19 per cent. In this series, the O55, O86, and O26 antigens were no more reactive than the filtrates from nonpathogenic strains. Sensitization with other filtrates and an evoking dose of O111 filtrate resulted in 37 per cent of positive reactions. Sites sensitized with other than O111 filtrates, and with non-O111 evoking antigen, gave the lowest rate of reactions. Only 14 per cent of such tests were positive. These figures include all animals in which some degree of reaction occurred; completely refractory rabbits were not included in the totals. In each animal both O111 and other antigens were tested.

Similar comparisons were made with filtrates of five strains of *E. coli* O127, six of O55, five of O26, three of O86, and two of O126. Results are summarized in TABLE 3. In these tests the heterologous comparisons did not, for the most part, include filtrates of enteropathogenic types. With O127, homologous filtrates reacted in 76 per cent of the tests. With O26 homologous filtrates, 82 per cent of the tests were positive, and with O126 (with only 2 strains compared), 60 per cent of positive reactions occurred. In contrast to this reaction pattern, neither O55 nor O86 reacted with homologous antigen to a higher degree than was the case with heterologous combinations. Reactions of heterologous combinations for O127, O26, and O126 were in each instance relatively low when the evoking injection was a nonpathogenic filtrate. With 0127, sensitization with heterologous filtrate and an evoking injection of O127 resulted in an increased rate of reaction, while with O26 and O126 both heterologous combinations were significantly lower than the homologous pairs.

Two essentially different reaction patterns were thus demonstrated among the six types of enteropathogenic *E. coli* studied. Among O111, O127, O26, and probably O126, the capacity of culture filtrates to sensitize for and to evoke the Shwartzman reaction was far higher for homologous serotypes than was the case with the group of cultures of nonpathogenic strains tested in parallel. Among the strains studied, the incidence of positive reactions was relatively low when enteropathogenic culture filtrates were used as sensitizing an-

TABLE 3

CUTANEOUS SHWARTZMAN REACTIONS WITH CULTURE FILTRATES OF *E. COLI* O127, O55, O26, O86, AND O126: HOMOLOGOUS AND NONENTEROPATHOGENIC FILTRATES

Type	Strains	Sensitized with filtrate	Evoking antigen	Total tests	Positive reactions	
O127	5	O127	O127	80	61	76%
O127	5	O127	Other*	32	9	27%
O127	5	Other*	O127	30	13	43%
O55	6	O55	O55	90	16	18%
O55	6	O55	Other*	43	6	14%
O55	6	Other*	O55	51	11	22%
O26	5	O26	O26	38	31	82%
O26	5	O26	Other*	20	16	20%
O26	5	Other*	O26	24	7	29%
O86	3	O86	O86	24	3	14%
O86	3	O86	Other*	15	3	20%
O86	3	Other*	O86	18	3	17%
O126	2	O126	O126	20	12	60%
O126	2	O126	Other*	12	3	25%
O126	2	Other*	O126	14	4	29%

* Includes O6, O5, O4, O3, and 8 nontyped *E. coli* strains from normal infants.

tigens and filtrates from nonpathogenic strains were used as evoking antigens. With the reverse combination, *i.e.*, with nonpathogenic strain filtrates as sensitizing antigens and enteropathogenic *E. coli* filtrates as evoking antigens, a significantly higher proportion of positive reactions was noted in the case of O111 and O127 filtrates. When filtrates of types O26 and O126 were similarly used, however, no essential difference between the two heterologous reacting combinations appeared, nor were differences noted when strains of types O55 and O86 were similarly tested.

The scope of this investigation has not yet permitted a detailed characterization of the reacting substances in the culture filtrates studied, but preliminary observations have revealed some pertinent attributes. When filtrates were stored at 5° C. for 10 months the potency of the reacting factors was noticeably diminished in the case of 8 strains of type O111. The proportion of positive reactions obtained with these filtrates when tested after storage was approximately one third that obtained when the filtrates were fresh, while fresh filtrates of the same strains, subsequently prepared, were as active as the original preparations had been when first tested.

Heating of filtrates of four strains of O111 for two hours at 100° C. caused a marked decrease in reactivity for the Shwartzman reaction. Since the endotoxin of *E. coli* has long been known to be thermostable, this change adds further weight to the suggestion that the reactivity described may not be due solely to such endotoxin.

In tests with four enteropathogenic and two nonpathogenic filtrates, the reacting factors were not removed by dialysis through cellophane against distilled water. Boivin-type antigens were prepared, by treatment with trichloracetic acid, with three strains of O111 and two nonpathogenic strains.[10] All of these antigens showed the capacity for inducing positive Shwartzman reactions. There was no difference, however, in the proportion of reactions between the two groups of antigens, in contrast to the preponderance of reactions that occurred with O111 culture filtrates of the same strains.

Three strains of *E. coli* O111 were subcultured on carbohydrate-free agar until relatively translucent and flat colonies were recovered These cultures, regarded as containing diminished amounts of K antigen, were used to prepare filtrates. These preparations were markedly less potent in inciting local tissue reaction than were filtrates of the same strains when maintained in optimal condition for production of K antigen by serial transfer on glucose agar.

Discussion

The enteropathogenic serotypes of *E. coli* occupy a unique position among enteric pathogens in that they are part of a large group of a biologically distinct species, distinguishable from nonpathogenic *E. coli* only in their capacity to cause infant diarrhea. In view of this circumstance, the pathogenesis of these organisms seemed best to be studied by comparing the enteropathogenic with the "normal" *E. coli* strains.

A true infection of laboratory animals with enteropathogenic *E. coli* is probably not attainable. In a comprehensive study of the action of *E. coli* strains on mice, Sjostedt attributed death of the animals solely to toxic action. Fecal and urinary strains were both found to be much more toxic when K antigen was present in an organism. These observations did not include the enteropathogenic types. In our toxicity tests, we did not establish existence of a clear-cut distinction between enteropathogenic and nonpathogenic strains in mice. The enteropathogenic strains tested all had B antigen present. The L.D.$_{50}$ differed for different serotypes but the variations more resembled a gradient of increasing toxicity not associated with specific serotypes. The surface antigens may well play a role in toxicity with this group of enteropathogenic strains, but the tests employed did not show it.

Tissue cultures have been used to study the growth of bacteria in a more complex and seminatural habitat, and the enteropathogenic *E. coli* were tested in this manner. Frequently tissue cultures were found to survive the presence of coliform bacteria for 18 hours or more and, in some instances, marked variations were noted in the ability of serotypes to proliferate in the tissue cultures. Nonpathogenic forms may differ from enteropathogenic serotypes in that the latter are able to proliferate under conditions where growth of the former is suppressed.

The occurrence of a generalized Shwartzman reaction in humans has been noted by Zuelzer[11] and by McKay.[12] A similar interpretation was made by Belnap and O'Donnell[13] on the basis of infants dying in an outbreak of infant diarrhea due to *E. coli* O111. Experimentally, hemorrhagic reactions of the Shwartzman type were demonstrated with culture filtrates of enteropathogenic *E. coli* strains of serotypes O111, O127, O126, and O26 to an extent far greater than was the case with several nonpathogenic strains. Strains of serotypes O55 and O86, however, resembled the nonpathogenic strains in this regard. The degree of type-specificity observed implied a relationship to the somatic antigen, but activity of filtrates was apparently greatest when cultures with large amounts of K antigen were used for production of culture filtrates.

The distinction between enteropathogenic and other *E. coli* serotypes, as to potentiality for inducing a Shwartzman-reactive state, may be either quantitative or qualitative. The study thus far made does not indicate a qualitative difference in the nature of the active substance and, since purified endotoxins of this group of organisms are essentially similar in behavior, the difference demonstrated may well reflect a difference in the amount or rate of formation of endotoxic material.

While the demonstration of a difference in production of Shwartzman reacting substance between some enteropathogenic and nonpathogenic strains of *E. coli* does not necessarily imply any causal relationship with the pathogenesis of infantile diarrhea due to *E. coli*, the distinctions noted suggest a possibility for further investigation.

Summary

The toxicity of enteropathogenic *E. coli* for mice was found to vary from that of nonpathogenic strains, but L.D.$_{50}$ values of the two categories overlapped markedly. Enteropathogenic *E. coli* may possess greater survival potential in tissue cultures than do nonpathogenic strains. A significant difference between the capacity of some enteropathogenic *E. coli* and of nonpathogenic strains to sensitize for and to evoke the Shwartzman reaction was demonstrated.

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EPIDEMIOLOGIC STUDIES ON ENTEROPATHOGENIC *ESCHERICHIA COLI* DIARRHEA

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Evidence that certain serologic types of *Escherichia coli* are causative agents of diarrhea, *i.e.*, gastroenteritis of infants has been accumulating in recent years. The first serotype was investigated by Bray[1] and by Bray and Beaven,[2] and the second serotype by Giles, Sangster, and Smith.[3] Kauffmann and Dupont[4] designated the antigenic structure of these strains as O111:B4 and O55:B5. Diarrhea associated with other serotypes, O26:B6, O86:B7, and O55:B5, has also been reported in more recent years.[5-7]

This paper presents the experience obtained on culturing microorganisms from cases of gastroenteritis admitted to the Children's Hospital of Pittsburgh in a four and one-half year period between October 1950 and August 10, 1955. Isolation of serotypes O111:B4 and O55:B5 was attempted for four and one-half years, the study being discontinued between October 1952 and March 1953 because of the infrequency of isolation of specific types. Isolation of the other serotypes was attempted as follows: O26:B6 for 21 months beginning on November 14, 1953; O86:B7 for 18 months beginning on March 11, 1954; and O127:B8 for 14 months beginning on June 11, 1954. The number of isolations of the five serotypes in the intervals given is presented graphically in FIGURE 1.

An epidemic of diarrhea associated with *E. coli* O111:B4 began in March 1953 in the Children's Hospital of Pittsburgh and was found to be related to a series of outbreaks in other institutions in the area.[8] A small outbreak of diarrhea associated with *E. coli* O55:B5 occurred in a hospital nursery outside of Pittsburgh, and the Children's Hospital of Pittsburgh received the two sickest infants. Other than these outbreaks, the cases of diarrhea associated with enteropathogenic *E. coli* were infrequent and sporadic. The incidence of isolation of *E. coli* O111:B4 and O55:B5 as related to the cases of gastroenteritis cultured at the Children's Hospital of Pittsburgh in a three-year period between October 1950 and March 1953 is presented elsewhere.[9] These data also include the incidence of isolation of the serotypes from control children adjudged not to have gastroenteritis, and the incidence of isolation of *Salmonella* and *Shigella*. TABLE 1 presents the same type of data for an additional one and one-half years, together with the incidence of isolation of the three additional serotypes O26:B6, O86:B7, and O127:B8 for varying intervals. TABLE 2 relates the incidence of isolation of five serotypes to the monthly admissions for gastroenteritis and is a continuation of similar data already reported for *E. coli* O111:B4 and O55:B5.[9] As shown by the tables in the previous and present reports, there is no striking seasonal effect on admissions.

In the four and one-half-year period of study, stool cultures were made on 575 cases of gastroenteritis and 1386 control subjects. From the 575 cases of gastroenteritis, 65 enteropathogenic *E. coli*, 81 *Salmonella*, and 1 *Shigella* were isolated. The incidence of enteropathogenic *E. coli* is weighted due to the

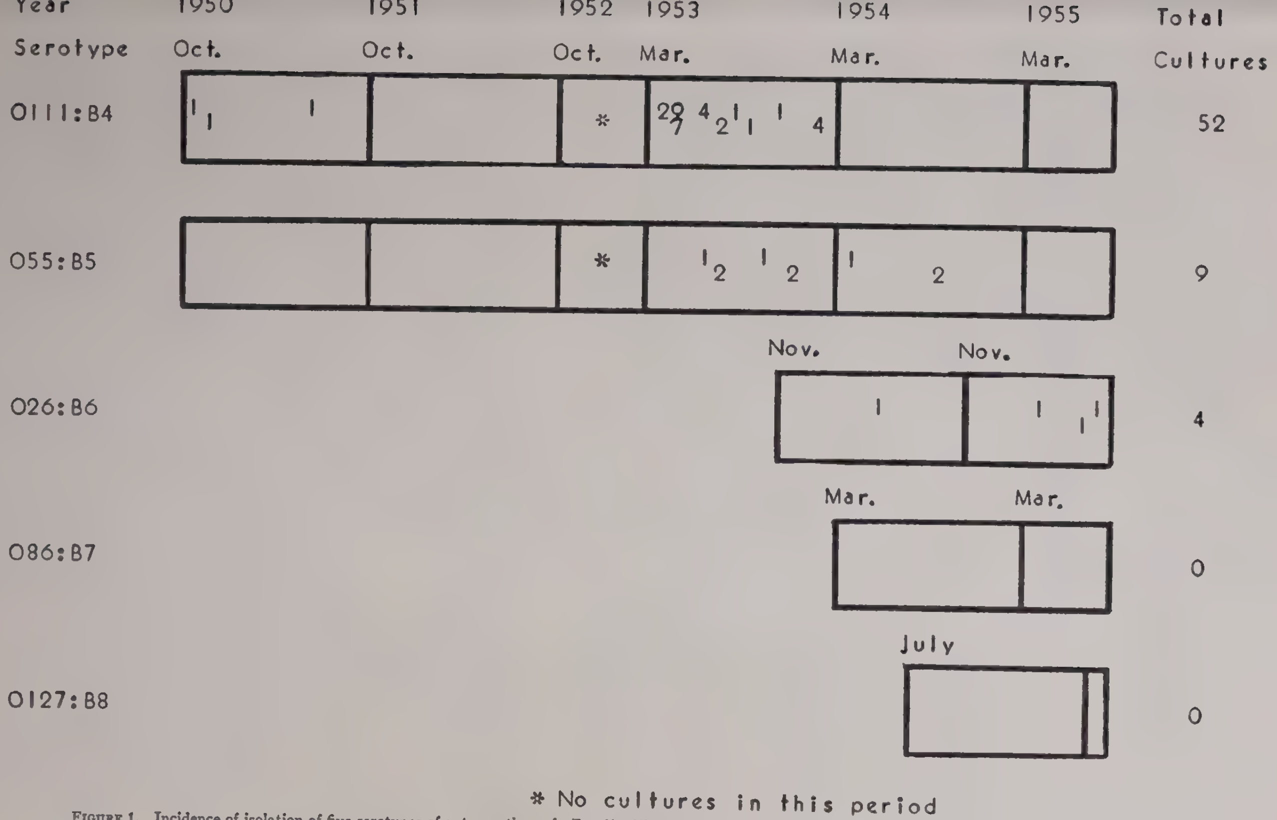

FIGURE 1. Incidence of isolation of five serotypes of enteropathogenic *E. coli*. Figures within blocks indicate numbers of specific *E. coli* found in each month.

TABLE 1

DATA RELATIVE TO THE INCIDENCE OF ISOLATION OF ENTEROPATHOGENIC *E. COLI*, *SALMONELLA*, AND *SHIGELLA*, MARCH 1954 TO AUGUST 1955

Year	No. cases gastro-enteritis	No. other patients cultured (controls)	Total patients cultured	Cases of gastroenteritis			Controls No. positive for *E. coli* B4-B8, *Salmonella*, or *Shigella*
				No. positive for *E. coli* B4-B8	No. positive for *Salmonella*	No. positive for *Shigella*	
Mar. 54 to Mar. 55....	134	477	611	5	16	0	0
Mar. 55 to Aug. 55.....	46	105	151	2	7	1	0
Totals..............	180	582	762	7	23	1	0

TABLE 2

DATA RELATIVE TO THE INCIDENCE OF ISOLATION OF ENTEROPATHOGENIC *E. COLI*

	1954										1955	
	M	A	M	J	J	A	S	O	N	D	J	F
No. cases gastroenteritis.........	13	7	6	5	7	6	13	13	12	7	19	18
O111:B4	0	0	0	0	0	0	0	0	0	0	0	0
O55:B5	0	1	0	0	0	0	2	0	0	0	0	0
O26:B6*	0	1	0	0	0	0	0	0	0	0	0	1
O86:B7	0	0	0	0	0	0	0	0	0	0	0	0
O127:B8	†	†	†	0	0	0	0	0	0	0	0	0
	1955											
No. cases gastroenteritis.........	16	11	7	13	3	4‡						
O111:B4	0	0	0	0	0	0						
O55:B5	0	0	0	0	0	0						
O26:B6	0	1	1	0	0	0						
O86:B7	0	0	0	0	0	0						
O127:B8	0	0	0	0	0	0						

* None found in November 1953 to March 1954.
† Not done in these months.
‡ To August 11, 1955.

epidemic previously cited. From the controls, no enteropathogenic *E. coli*, *Salmonella*, or *Shigella* were isolated.

The epidemic of 1953 associated with *E. coli* O111:B4 was brought to our attention on March 11 by the readmission to the Children's Hospital of Pittsburgh of three infants with severe diarrhea who had previously been in residence for other causes. In the course of time, 25 children were found to be involved in the hospital outbreak, divided according to residence on the infant wards (third floor) or contagious wards (eighth floor). The 20 children involved on the third floor included the 3 infants already mentioned, 1 infant who died suddenly, 5 infants found by a bacteriologic survey of some of the infants on the floor, 6 infants who were discharged to their homes and developed diarrhea, 1 infant who was cross-infected at a later date, and 4 infants who were involved in a secondary outbreak in April. On the eighth-floor wards there were 4 infants who were cross-infected from other patients on the floor, and a 5th who was cross-infected at home from a sibling who had been on the wards and was discharged home.

This outbreak was found to involve other institutions. Maternity Home A was investigated on March 18 after an outbreak of diarrhea in 12 infants that was attributed to an infant who had been in the Children's Hospital of Pittsburgh infant wards for erythroblastosis fetalis. A second outbreak at Home A, involving four infants, occurred in June. Early in March, an infant at Maternity Home A, not involved in the first or second outbreaks, was sent to Maternity Home B. This change was followed by diarrhea in 5 of 11 infants in Home B. In the first outbreak at Home A, an infant was sent to Maternity Home D, resulting in an outbreak of diarrhea involving 20 of Home D's 45 infants. Of these affected infants, three were sent to Hospital E, where eight infants developed diarrhea. In July, Hospital F had an outbreak of diarrhea in six infants, one of whom had been in Hospital E earlier. Finally, in this period there were two sporadic cases of diarrhea associated with *E. coli* O111:B4 that seemed to have no relation to the other outbreaks.

In the complete paper[8] alternate theories for the transmission of these infections from one institution to another have been presented.

A number of hospital outbreaks of diarrhea associated with *E. coli* O111:B4 have been documented,[9] and interhospital outbreaks associated with *E. coli* O111:B4 and O55:B5 have been reported by Rogers and Koegler.[10]

Summary

The incidence of five serotypes of enteropathogenic *E. coli* isolated from infants with gastroenteritis over periods varying from 14 months to four and one-half years has been presented. The history of an outbreak of diarrhea involving multiple institutions and associated with *E. coli* O111:B4 has been presented as evidence in favor of the pathogenicity of this serotype.

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SPREAD AND CONTROL OF *ESCHERICHIA COLI* DIARRHEAL DISEASE

By Warren E. Wheeler

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During the fall of 1952, an outbreak of gastroenteritis due to *E. coli* O111 occurred in the nurseries of the Children's Hospital, Columbus, Ohio. Positive stool cultures were obtained from 26 infant patients and 9 adult personnel cross-infected in the nurseries. Three other infants who had acquired this type of infection at home were admitted during this outbreak. The patients during the early days of the outbreak were treated with chloramphenicol, which succeeded in ameliorating the toxicity and reducing the mortality to zero.[1] In the dosage used (35 mg./kg./day), however, this drug was not uniformly successful in eradicating the pathogens from the stools. In many of the cases, drug-resistant strains of the organisms developed. Rigid aseptic nursery technique and prophylaxis of exposed babies with chloramphenicol failed completely to limit the epidemic.

For these reasons, neomycin sulfate* was substituted for chloramphenicol in a dose of 50 mg./kg./day. This drug controlled the toxic manifestations of the disease equally well, uniformly eradicated the organisms from the stool cultures, and in only one instance was its use followed by cultural relapse. In no case did neomycin-resistant strains of the organism develop.

Once we (my associates in this work and myself) began to use neomycin in the treatment of every case, the incidence of cross-infection on the ward ceased. We attributed this to the remarkable consistency with which negative stool cultures were obtained after one or two days of treatment. Contrariwise, we attributed our previous inability to prevent the spread of the disease, in spite of rigid aseptic technique, to the fact that in the acute phase the enteropathogenic *E. coli* are present practically in pure culture in the stools. Infants under the age of two months were considerably more susceptible to cross-infection than older babies.

An obstetrical nursery may be closed in order to stop an epidemic of diarrhea, but a nursery in a general children's hospital must be prepared to treat infants acquiring this disease at home. We knew that *E. coli* O111 was present in our community. We therefore set up an experimental plan that has been in operation for almost three years to demonstrate that it is possible to operate such a nursery without significant cross-infection from this and similar organisms. The plan assumes that enteropathogenic *E. coli* of all types are susceptible to neomycin treatment, and that the use of this drug will quickly reduce the number of infective organisms discharged in the stools.

We set aside one four-bed room on our diarrhea ward as a quarantine facility where all diarrhea patients under two years of age are admitted and treated pending the outcome of their admission stool cultures, three rectal swab cultures being made within the first six hours of entry. We presume each new arrival to be infected with enteropathogenic *E. coli* until proved otherwise. Isolation technique is rigidly enforced. Each patient is given neomycin sulfate in a dose of 50 mg./kg./day by mouth in four divided doses. On the day after admission, if the battery of admission stool cultures is reported negative, the patient is released from quarantine and removed to the general diarrhea unit. The neomycin is continued, however, for 48 hours. Subsequently, the patient receives stool cultures 3 times during the first week and thereafter as indicated. If admission stool cultures are positive for enteropathogenic *E. coli*, the patient is isolated in a separate room. Neomycin is continued until his discharge from the hospital or for at least 10 days. Other infants admitted to the quarantine room the same day as the patient with positive stool cultures are retained there for 24 more hours. Three more negative rectal swab cultures are required before such infants are released to the general diarrhea unit. During the first 2 years of our study, stool cultures were obtained on nursery personnel after each enteropathogenic *E. coli* patient was treated. During these 2 years we did not detect any carriers among the personnel, so we have now omitted this procedure.

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* Some of the neomycin used in this study was supplied by The Upjohn Company, Kalamazoo, Mich.

The restricted bacterial flora induced by neomycin alters the stool so that infants usually produce two to six rather small, greenish stools with mucus daily. The normal color, consistency, and number of stools usually returns within a day or so after discontinuing the drug. When neomycin is administered for more than two days, vitamin K is also given to prevent hypoprothrombinemia. No untoward effects have been noticed due to the two-day treatment with neomycin.

About 300 infants under 2 years of age are admitted annually to the Children's Hospital for the treatment of diarrheal disease. Since the recognition of enteropathogenic *E. coli* depends on the agglutination of selected colonies of bacteria in specific serum, it is obvious that many thousands of agglutination tests would require a large amount of diagnostic antisera. We have been in the beggar's position regarding sera and for diagnostic purposes have had to depend on gratuitous supplies of this scarce commodity. Rather early in the course of our plan of operation, Bruce Daily, our bacteriologist, noted that the enteropathogenic types of *E. coli* were inagglutinable by acriflavine, whereas practically all of the nonenteropathogenic strains were promptly agglutinated by this dye. Since May 1953, therefore, we have modified the usual diagnostic procedure by testing each admission stool culture for agglutination with acriflavine, resorting to the use of sera only on those strains that are not promptly and coarsely agglutinated by the dye. We test with sera the obviously acriflavine-negative colonies and those that demonstrate any fine granularity. About one third of such colonies have proved to be strains of enteropathogenic *E. coli*. We have no information on how many enteropathogenic strains we may have missed by this technique, but can report that we have not had any epidemic diarrhea in our ward. In a few instances where babies appeared to have clinical relapses of diarrhea, we have attempted to agglutinate the acriflavine-positive organisms obtained from these babies with polyvalent sera and have uniformly failed. Other workers, notably Joan Taylor in London, England, William Ferguson of the Michigan Department of Health, Lansing, Mich.,

TABLE 1

INCIDENCE OF DIARRHEA DUE TO ENTEROPATHOGENIC *E. COLI*—CHILDREN'S HOSPITAL, COLUMBUS, OHIO, 1953 TO 1955

The recognition of enteropathogenic *E. coli* as causative agents in infants with diarrheal diseases has increased as more types of diagnostic antisera have become available. It would appear that formerly frequent types of *E. coli* may be on the wane in this community.

	Cross infections	O111	O55	O26	O86	O119	O125	O126	O127	Total
1952 (Epidemic)	26	3+								29+
1953	4	5								9
1954	2	6	7	8	1	0	0	2	0	26
1955	0	0	2	0	0	1	1	4	1	9

and William Ewing of the Communicable Disease Center, Atlanta, Ga., had made prior observations on the general acriflavine-inagglutinability of these types of *E. coli*.

TABLE 1 illustrates the incidence of disease due to enteropathogenic *E. coli* admitted to the Children's Hospital, Columbus, Ohio, since our epidemic in 1952. During 1953, when we had available to us only O111 diagnostic serum, the number of cases admitted to the hospital was relatively small. In January 1954 we obtained from W. Ferguson a trivalent antiserum against *E. coli* O111, O55, and O26. The use of this serum was immediately followed by an increase in the strains isolated. In October 1954 a polyvalent serum for the newer types of enteropathogenic *E. coli*, supplied by Cyril Stulberg of the Children's Hospital in Detroit, Mich., enabled us to detect patients infected with organisms of these types. In all probability, during 1953 and part of 1954, we had admissions to our wards of patients suffering from specific *E. coli* enteritis due to some of the newer types and perhaps to some types that have not yet been discovered. All of the types that we have isolated have responded quite well to neomycin, the usual result being a disappearance of positive culture on the first or second day. On only two or three occasions have positive cultures continued to the third day, and then the enteropathogenic *E. coli* were present in small numbers. It must be emphasized that during this time the diarrhea nursery has been scrutinized very carefully from the standpoint of clinical cross-infection, and no significant incidence of relapsing diarrhea or obvious cross-infection has been observed.

Soon after the beginning of our plan for the prevention of cross-infections, four instances of diarrhea or relapse of diarrhea occurred in babies who had had previous negative stool cultures. These appeared during a two-week period. Two occurred on an overflow ward for recovering diarrhea patients, one on a ward for babies with respiratory disease that was serviced by personnel from the diarrhea ward at some hours of the day, and one on the diarrhea ward. These, of course, might be considered missed diagnoses on the basis of the original cultures. On the other hand, they can also be considered cross-infections and, for the purposes of this presentation, we so consider them. Attempts to find a carrier among the personnel at that time were not successful but, from the location of the patients at that time, we must assume that some of our personnel were responsible. During 1954 there were also 2 instances of probable cross-infection. During these 3 years we have treated approximately 1000 infants under 2 years of age with diarrheal disease in this hospital and have encountered 37 infants with gastroenteritis due to enteropathogenic *E. coli*. During this same period, 6 patients developed diarrhea on the basis of cross-infection, but there was no outbreak approaching epidemic proportions.

While we have been unable to assemble convincing statistics on the matter, it has been quite obvious to observers of the diarrhea nursery that relapsing diarrhea, which used to be fairly frequent on our wards, has become almost a thing of the past. Looking back, it seems obvious that we must have been dealing with this type of diarrhea for many years, and that cross-infections on our ward must have been really quite common. We considered such instances "relapses" and took refuge in the clinical dictum that infantile diarrhea is apt to be a relapsing disease and that, with each additional relapse, the patient is more likely to die. Our results in the past three years make us now feel quite differently, and we now claim that diarrheal disease in infants, other than that caused by enteropathogenic *E. coli*, is rarely relapsing and that, probably, instances of clinical relapse really mean cross-infection or superinfection of the infant with a new diarrhea-producing agent.

Based on these experiences, we have modified the usually practiced and advocated plan for the management of epidemic disease for use in newborn nurseries. Very satisfactory results were obtained by using this modification during an epidemic of impetigo due to a highly pathogenic strain of *Staphylococcus* at the University and Children's Hospitals in Columbus. This indicates that the principles involved can be applied to other epidemic bacterial diseases in the newborn period equally well. Our present concept emphasizes that isolation technique can be relied on only to hinder and delay the colonization of infants by *Staphylococcus* and *E. coli*. "Technique" cannot, however, ensure that infants will remain free of such organisms indefinitely. If a baby can become colonized with "normal" *E. coli* and *Staphylococcus* in spite of technique in the absence of an epidemic, he stands every chance of acquiring a disease if pathogenic strains of these organisms are introduced into the nursery. When an incipient epidemic starts in the nursery the problem therefore must be attacked from a different point of view; namely, that of the susceptibility of the infant.

Fortunately, both diarrhea and impetigo are bacterial in origin, and the causative organism is usually found to be susceptible to one of the antibiotics. In the case of *E. coli*, the pathogenic strains so far studied have been sensitive to neomycin. The situation is more complicated in the case of *Staphylococcus*, since most strains carried by hospital personnel are penicillin-resistant.

The organism causing an impetigo outbreak must therefore be isolated and tested to find an effective drug. Once it has been found, however, recent epidemics have been terminated immediately when the drug has been given to the entire susceptible baby population of the affected nursery. By this procedure, babies have been temporarily converted to a population of non-susceptibles. This provides a period of time that can be used to identify the organism further, to search for carriers among the personnel, to plug any

Table 2

Control of Infection in Nursery for Newborn or Premature Infants

This is a summary of the recommendations of the Committee on the Fetus and Newborn of the American Academy of Pediatrics.[9] The items in italic type are the additions that we would make. This applies to the two greatest epidemic diseases of the newborn—*E. coli* diarrhea and impetigo—both of which are bacterial in origin.

	Allocation	Culture & treatment
Original patient	Discharge from maternity unit immediately	Culture *and treat immediately*
Patient's mother	*Isolate*	*Culture, treat if positive, or if baby positive*
Nursery contacts and nursery personnel	Retain in original nursery	Culture *and treat if original patient positive*
	Reopen nursery only after being evacuated and cleaned	*Keep testing for development of antibiotic resistance*
New patients	New nursery with new personnel	*Culture frequently*
	Close down if cases appear in new nursery	

loopholes in technique, to clean the nursery, and to ensure eradication of the organism from the nursery population. Once these measures have been accomplished, the antibiotic umbrella may be lowered and a "normal" state resumed. In the meantime, a serious epidemic may have been averted.

Table 2 depicts a summary of the recommendations of the Committee on the Fetus and Newborn of the American Academy of Pediatrics,[2] Evanston, Ill. The items listed in italic type are modifications that we would add to this currently accepted plan. Our conviction is that effective antibiotic therapy given to patients can shorten the time during which they discharge large numbers of pathogens and that antibiotics given to contacts can prevent colonization of otherwise susceptible infants. With this assistance from properly chosen antibiotics, it seems foolish to rely entirely upon technique to prevent the spread of diseases that in the past have spread in spite of good technique.

Summary

(1) An experimental plan to prevent the spread of enteropathogenic *E. coli* in a diarrhea nursery in a children's hospital has been in operation for three years.

(2) The plan is based on the following principles: (a) minimizing the colonization of uninfected infants with pathogenic organisms by the practice of good isolation technique; (b) prompt reduction of the numbers of pathogenic organisms excreted by incoming patients by the use of neomycin; and (c) temporary conversion of incoming patients with other types of diarrheal disease to nonsusceptibles by the use of neomycin.

(3) During the 3 years of operation of this plan over 1000 diarrhea patients

have been treated. Thirty-seven of these have had enteropathogenic *E. coli* in their stools. Six cross-infections have occurred.

(4) The plan has been modified for use in nurseries for the newborn in event of the occurrence of epidemic diarrhea of the newborn or epidemic impetigo.

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Discussion of the Paper

IRVIN OLITZKY (*Philadelphia Department of Health, Philadelphia, Pa.*): In our hands, acriflavine agglutinates our *E. coli* of type O127. Since the acriflavine inagglutination has been claimed to be a flagellar phenomenon, and since *E. coli* O127 is nonmotile, may this not be a source of error?

WARREN E. WHEELER: In our hands and in Cyril Stulberg's, *E. coli* O127 is acriflavine negative, as are the other types, so that in this instance, I should doubt that the reactions depend on flagellar antigens. The difficulty in recommending this test is that it depends on an empiric observation. Until we know why some bacteria are agglutinated and others are not, we cannot explain why the test may be more or less specific in some hands and not in others. Various lots of acriflavine are known to give slightly different responses. The matters of pH and electrolyte concentration may also affect the results. The way we perform the test in our laboratory is to make a heavy suspension of a colony on a slide in a drop of normal saline solution. Once it is obvious that the bacteria are not salt-agglutinable, one drop of a 1:500 solution of neutral acriflavine in distilled water is added, and the reaction is observed for no longer than one minute. Positive agglutination usually begins in a matter of seconds and, with clearly acriflavine-positive colonies, the agglutination is complete in one half or three quarters of a minute. If nonagglutinated bacteria remain suspended in the drop at the end of a minute, we go ahead and type the colony with serum. I merely claim that in our hands, for reasons we do not understand, it has been a satisfactory screening procedure.

MERLIN COOPER (*Children's Hospital Research Foundation, Cincinnati, Ohio*): I am a bit worried about the dose of neomycin used here. As I reported earlier in this meeting, we did not obtain consistently negative stool cultures using this or even higher doses after we discontinued treatment. We now recommend 150 mg./kg./day of neomycin for 2 days and 100 mg./kg./day for 8 more days.

WARREN E. WHEELER: I am glad Merlin Cooper brought out this point. We were so impressed with the contagion when *E. coli* O111 was involved that we never removed patients from treatment while they were still in the hospital. I am sure Cooper is right and, if one wants to ensure negative cultures after treatment has been stopped, one must use a higher dose than we use, a longer period of treatment, or both. Our data merely show that we have had consistently negative cultures while patients were still receiving 50 mg./kg./day.

CONTROL OF EPIDEMIC DIARRHEA OF THE NEWBORN IN HOSPITAL NURSERIES AND PEDIATRIC WARDS

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As a cause of death during the neonatal period, epidemic diarrhea of the newborn is relatively unimportant. Yet hospital outbreaks of this disease, when they do occur, are serious affairs. Like all diseases that descend "on the people," they are frightening. When these diseases descend on the hospital, a scientific institution surrounded by all the aura and prestige of modern medicine, their danger appears magnified and publicity is inevitable.

It is our intention to summarize nine years' experience with hospital nursery outbreaks of diarrhea of the newborn in upstate New York, to discuss the rationale of hospital control measures that arises out of this experience, and to describe our program for their prevention and control.

Nursery outbreaks in upstate New York. In upstate New York, epidemic diarrhea of the newborn has been known to exist since 1935. After the disease became reportable in July 1946, more reliable information became available. TABLE 1 shows the number of reported cases, deaths, and outbreaks of diarrhea in infants under 28 days of age from July 1946 through June 1955. This includes sporadic as well as epidemic cases and deaths. We have considered an outbreak to be three or more cases occurring over a one-month period in the same nursery. Our experience, of course, is limited to those outbreaks which come to the attention of public health authorities. All of the outbreaks in 1954 and 1955 were reported following the publicity given to a serious epidemic in a hospital in eastern New York State.

A review of the 35 reported outbreaks revealed several interesting facts. The over-all case fatality rate was 11.8 per cent. In 23 outbreaks there were no deaths while, in 12 others, the case fatality rates ranged from 7.2 to 80 per cent. TABLE 2 shows that outbreaks occurred with considerably greater frequency in large hospital maternity services than in small ones. The number of cases per outbreak was also related directly to the size of the maternity service. These relationships are to be expected since, in a large maternity service, the infant is likely to be handled by a greater number of people than in a small one, and the group endangered is a larger one. It is also possible that outbreaks are more likely to be reported to the health department when they occur on large maternity services. The case fatality rate in these 35 outbreaks did not vary significantly with size of maternity service (TABLE 3).

TABLE 4 illustrates the importance of prompt, vigorous action to control outbreaks. Too often no report was made to the health department until one or more deaths had occurred. In these situations, record review and discussion with the staffs clearly indicated that diarrheal disease had been smoldering in the nurseries for several weeks or months prior to its being reported. This relationship between delay in reporting and deaths is to be expected, since the

TABLE 1

DIARRHEA OF NEWBORN—UPSTATE NEW YORK REPORTED CASES, DEATHS, AND HOSPITAL NURSERY OUTBREAKS, JULY 1946 TO JUNE 30, 1955

Year	Cases*	Deaths†	Nursery outbreaks‡
1946	75	18	5
1947	203	47	6
1948	160	27	5
1949	68	14	2
1950	67	19	3
1951	56	3	5
1952	7	5	0
1953	17	9	0
1954	104	21	5
1955	41	3	4
Total................	798	166	35

* Includes sporadic cases.
† In 1946 through 1951, figures include deaths not primarily attributed to diarrhea.
‡ Six outbreaks are recorded in 1942 and 7 in 1945.

TABLE 2

HOSPITAL NURSERY OUTBREAKS OF DIARRHEA OF NEWBORN, BY SIZE OF MATERNITY SERVICE, UPSTATE NEW YORK, JULY 1, 1946 TO JUNE 30, 1955

Size of maternity service (No. of births per year)	Total number of hospitals in upstate New York, 1950	Number of outbreaks	Number of outbreaks per 100 hospitals per year	Number of cases	Number of cases per outbreak
0–99	39	1	0.3	3	3.0
100–499	88	6	0.8	40	6.7
500–999	59	10	1.9	136	13.6
1000 and over	46	18	4.3	319	17.7
Total................	232	35	1.7	498	14.2

TABLE 3

CASE FATALITY RATES IN 35 HOSPITAL NURSERY OUTBREAKS OF DIARRHEA OF NEWBORN BY SIZE OF MATERNITY SERVICE, UPSTATE NEW YORK, JULY 1, 1946 TO JUNE 30, 1955

Size of maternity service (No. of births per year)	Cases	Deaths	Case fatality rate per 100 cases*
0–99	3	0	0.0
100–499	40	6	15.0
500–999	136	18	13.2
1000 and over	319	35	11.0
Total....................	498	59	11.8

* Number of deaths per 100 reported cases.

TABLE 4

DEATHS IN 35 HOSPITAL NURSERY OUTBREAKS OF DIARRHEA OF NEWBORN BY DELAY IN REPORTING OF OUTBREAK TO HEALTH DEPARTMENT, UPSTATE NEW YORK, JULY 1, 1946 TO JUNE 30, 1955

Interval between onset of first case and reporting of outbreak to health dept.	Number of outbreaks	Number of cases	Number of deaths	Number of deaths per outbreak
One week or less	12	102	4	0.3
Eight days to 1 month	12	174	22	1.8
More than 1 month	10	216	33	3.3
Unknown	1	6	0	0.0
Total	35	498	59	1.7

outbreaks reported late were of longer duration. One cannot escape the inference, however, that if the outbreaks had been reported sooner and action had been taken to control them, fewer cases and fewer deaths would have occurred.

No microorganism could be implicated as the etiologic factor in 27 outbreaks. Two outbreaks were probably caused by *Salmonella*, while another was characterized by the finding of large numbers of *Staphylococcus aureus* and *S. albus* in the stools of many cases. In five outbreaks, enteropathogenic *E. coli* were isolated from the stools of two or more patients and are believed to have been the causative agents. *E. coli* O111:B4 was isolated in association with three outbreaks, and O127:B8 and O26:B6 in association with one each. The low incidence of enteropathogenic *E. coli* is deceiving, since a thorough search for this microorganism was made in very few of the 35 outbreaks. Frozen stool specimens from 2 epidemics examined 5 years later revealed enteropathogenic *E. coli*.[1] We have been impressed by the frequency with which these microorganisms have been found when looked for during the past 2 years. The severity of the outbreaks with probable enteropathogenic *E. coli* etiology is striking. The case fatality rate for 139 infants involved in 5 outbreaks in which enteropathogenic *E. coli* were isolated was 28 per cent as compared to a case fatality rate of 6 per cent for 359 infants in the remaining 30 outbreaks. This contrast may be somewhat overstated, since a more thorough search for the etiologic agent was made in the more serious outbreaks. All but 1 of the deaths associated with enteropathogenic *E. coli* outbreaks occurred before neomycin was shown to be effective against these microorganisms.

Approximately 200 cultures of *E. coli* isolated in local laboratories and 500 fecal specimens submitted directly to the Division of Laboratories and Research were examined in the latter institution between July 1, 1954, and September 1, 1955. The source of material included infants and children with diarrhea and a disproportionately larger number of exposed infants and associated nursing and other hospital personnel. Stools from 28 individuals were shown to contain enteropathogenic *E. coli* as shown in TABLE 5. All of this latter group had diarrhea and, with the exception of a child of 6 years, all cases were in infants under 2 years of age.

The variation in severity and character of different outbreaks points almost

TABLE 5

ENTEROPATHOGENIC *E. COLI* IDENTIFIED IN THE DIVISION OF LABORATORIES AND RESEARCH, JULY 1, 1954 TO SEPTEMBER 1, 1955

Serotype	Number of patients		
	Sporadic cases	In hospital outbreaks	Total
O111:B4	6	2	8
O26:B6	5	2	7
O55:B5	5	0	5
O127:B8	1	2	3
O126:B16	2	0	2
O125:B15	2	0	2
O111:B4 and O26:B6	1	0	1
Total	22	6	28

certainly to a variety of etiologic agents. The clear association of several outbreaks with a high prevalence of diarrhea in the community, and often in the medical and nursing staff caring for the infants, suggests that the causative microorganisms in some hospital outbreaks are the same as the microorganisms causing diarrhea in the community.

Source and spread of nursery outbreaks. FIGURE 1 represents an attempt to diagram the potential sources of a hospital nursery outbreak and the channel through which infection may be introduced by such sources. Although in some of the outbreaks investigated we have arrived on the scene too late to implicate a source, all of the sources illustrated in the diagram have been clearly implicated alone or in combination.

FIGURE 2 represents an attempt to illustrate the channels through which infection may be spread once it has been introduced into the nursery for the newborn. Here we feel that we are on firmer ground, since we have yet to encounter an outbreak in which these channels of spread were not wide open at the time we arrived on the scene. It is this observation that leads us to believe that hospital outbreaks are preventable if proper nursery policies and techniques are observed.

These diagrams presuppose that epidemic diarrhea of the newborn is spread by the fecal-oral route and is neither air-borne nor spread by droplet infection. It is unlikely that hands of nursing personnel contaminated with a pathogenic microorganism can be washed clean of every last pathogen in one or two minutes at the scrub sink. Therefore, a second hypothesis on which control measures are based is that relatively large numbers of pathogens must be ingested to produce disease. It is unfortunate that so little documentation exists to support this hypothesis. Ferguson's experiments in feeding enteropathogenic *E. coli* to volunteers,[2, 3] Gordon's virus-feeding experiments,[4] and other work cited by Ferguson elsewhere in these pages indicate that dosage was a factor in producing symptoms of diarrheal disease in adults. Perhaps these findings apply also to infants.

Principles of prevention and control. Unless babies are handled with complete surgical asepsis at all times (a practice which is neither practical nor psychologically desirable) it is impossible to bar completely the access of an infective agent to the nursery for the newborn. Certain sources or channels of introduction, however, can be completely controlled (FIGURE 1). The terminal heating of feeding units (provided such terminally heated units are the only means by which a baby is fed) is a guarantee that infective agents will not be introduced by this route. The elimination of all common equipment and the care of all babies in their own bassinets with their own individual equipment eliminate this potential source. A hospital policy that ensures that no infant born outside of the hospital enters the nursery, under the assumption that such an infant may harbor an infective agent, eliminates this third source.

Mothers and the variety of hospital personnel handling infants remain as two potential sources of infection that can never be completely controlled. Hospital policies that prohibit a baby born to a mother with diarrhea from being placed in the normal nursery for the newborn may help to eliminate an obvious source of infection. Similarly, a policy that prohibits hospital person-

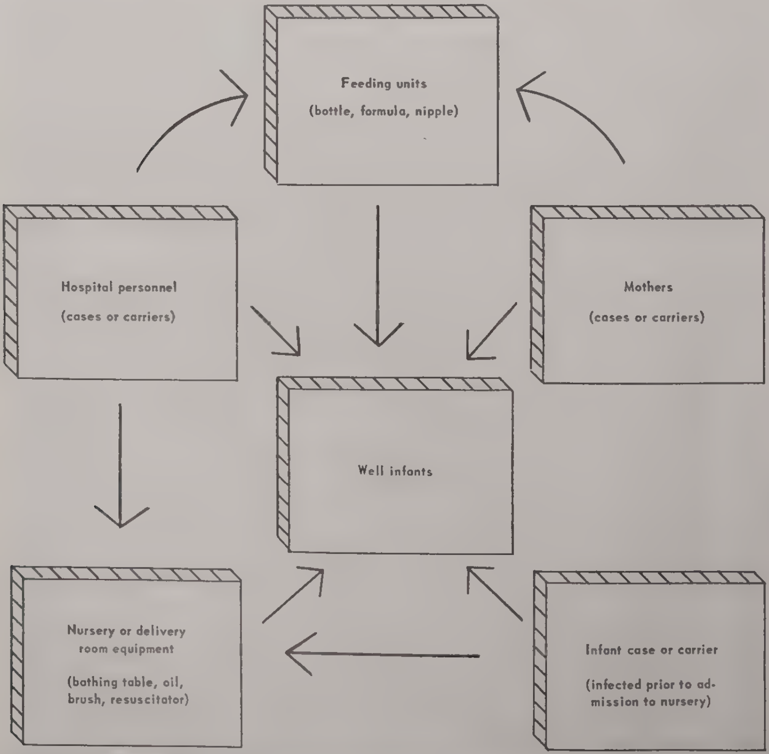
Feeding units
(bottle, formula, nipple)
Hospital personnel
(cases or carriers)
Mothers
(cases or carriers)
Well infants
Nursery or delivery room equipment
(bathing table, oil, brush, resuscitator)
Infant case or carrier
(infected prior to admission to nursery)

FIGURE 1. Sources and channels through which infection may be introduced into a nursery for the newborn.

nel exhibiting symptoms of illness from working with normal newborn infants may help to eliminate this source.

It is perfectly obvious that neither of these policies, however, can be 100 per cent effective. If outbreaks are to be avoided, therefore, a mother and baby must be considered as a single unit to be isolated from other such units. Each baby must be handled at all times on the assumption that he is a potential source of infection (FIGURE 2). It is unlikely that direct-contact spread between infants occurs very frequently, but we have come across situations in which two infants have shared an incubator or bassinet. Furthermore, crowded quarters and the use of a common infant carrier predispose to spread by inviting common care without hand washing.

The control of nursery personnel as a channel of spread embraces such factors as the provision of adequate work facilities, the assignment of a trained and properly supervised staff in sufficient quantities to meet the nursery needs, and the elimination of useless nursery routines such as frequent temperature taking, excessive bathing, *etc.* Only under such circumstances can the nursery staff be expected to carry out hand-washing and other techniques that isolate each mother-baby unit from every other mother-baby unit. Control measures depend also upon competent, interested medical and nursing supervision, and a hospital administration that understands that the full needs of the maternity service must be met without compromise.

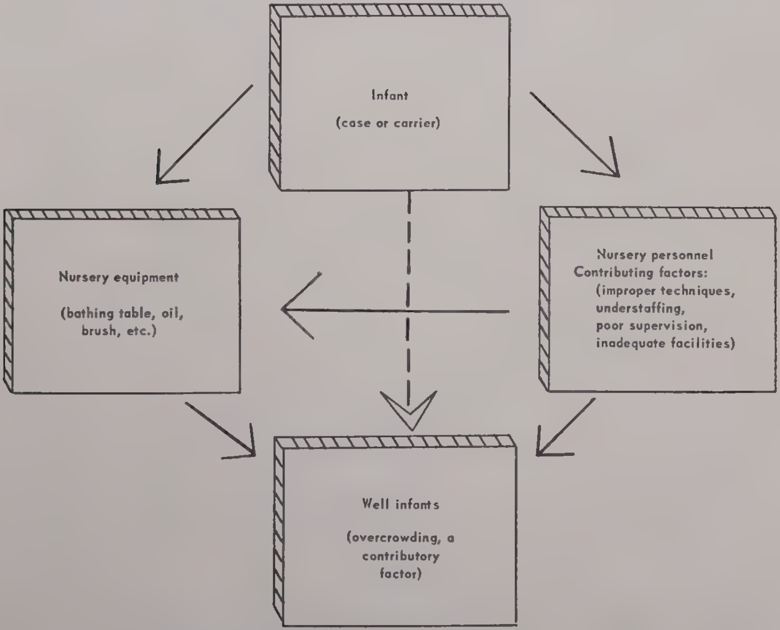
Infant
(case or carrier)
Nursery equipment
(bathing table, oil, brush, etc.)
Nursery personnel
Contributing factors:
(improper techniques, understaffing, poor supervision, inadequate facilities)
Well infants
(overcrowding, a contributory factor)

FIGURE 2. Channels through which infection may spread in a nursery for the newborn.

An observation nursery provides an additional safeguard. Such a separate unit should not be used to isolate diagnosed cases of disease. Its purpose is to allow quick segregation (without specific medical authorization) of any infant suspected of being ill. If a definite diagnosis of infection is made, the infant should be removed immediately from the maternity floor and isolated on another service.

Obviously these same principles of control should assist in preventing the introduction and spread of any pathogen using the same channels, whether it incites diarrhea in its host or some other disease, such as impetigo.

When an outbreak of diarrhea occurs in the nursery, the control measures put into effect are nothing more than a tightening of the barriers that have already been discussed. In most instances it suffices to consider as potentially infected all infants who have been housed in the same nursery unit as the infected infants. Newborn infants from the delivery room should be admitted to other nursery units and cared for by other personnel. The use of antibiotics, particularly neomycin, has been helpful in controlling outbreaks caused by enteropathogenic *E. coli*. Occasionally it has been necessary to close the entire nursery service. Where this has been necessary, however, the reasons have been the failure of nursery personnel to carry out proper techniques or extremely inadequate existing facilities. Closure under these circumstances has permitted us to work with the hospital personnel until they practiced proper techniques, or has permitted the hospital to remodel its nursery facilities.

Our experiences with hospital outbreaks of diarrhea have ranged from small hospitals to large ones with excellent reputations. In every case the channels of spread were very obvious to an outside observer. Most often, observation was far more productive during the small hours of the night than during the bright daytime hours.

Enforcement of control measures. Laws and regulations to improve social welfare and public health can be effective only if they represent an expression of common concern and if they are fairly and firmly administered. Public agencies administering regulations for nurseries for the newborn owe a great debt of gratitude to the American Academy of Pediatrics, Evanston, Ill., whose Committee on the Fetus and Newborn has issued two admirable statements of acceptable nursery policies and practices.[5] Without these authoritative statements it is unlikely that laws and regulations would be possible or enforceable. With such a statement available, however, we have found ourselves in the position of being requested to enact and enforce regulations by the very groups that are to be regulated. Under these circumstances, the firm enforcement of regulations becomes a positive effort to improve the health and the welfare of the community rather than an instrument of repression. When our regulations were revised this fall, they were welcomed by physicians and hospital administrators as a tool to help them achieve gains that they might have not been able to achieve without the force of law behind them. We have often suggested that plans for construction of new nurseries be revised to include what we felt were essential elements. Occasionally we have insisted on revision over the strenuous objections of architects and administrators. We have invariably been gratified, after construction was completed, by learning that even those

TABLE 6
INFANT LIVES SAVED FROM 1950 TO 1954 AS A RESULT OF THE DECLINE IN THE WEIGHT-SPECIFIC NEONATAL MORTALITY RATES BETWEEN 1945 TO 1949 AND 1950 TO 1954

Birth weight (grams)	Infant deaths		Number of lives saved	Per cent decrease in deaths
	Actual deaths, 1950–1954	Deaths expected under 1945–1949 rates		
1000 or less	3,995	4,022	27	.67
1001–1500	2,587	3,195	608	19.03
1501–2000	2,033	2,667	634	23.77
2001–2500	1,625	2,354	729	30.97
2501 and over	4,403	6,475	2,072	32.00
Total..................	14,643	18,713	4,070	21.75

who had opposed our suggested changes were now delighted that we had insisted upon them.

Responsibility for interpretation and enforcement of regulations for nurseries for the newborn in New York State is placed in the hands of local full-time health authorities. The devotion and skill of county, city, and district public health physicians and nurses account for whatever success these regulations may have had in improving care of the newborn in New York State, illustrated by TABLE 6. We believe decentralization of authority accomplishes far more in the long run than authority retained in a state agency far removed from the local scene. At the same time it poses certain disadvantages relative to the training and experience of local public health personnel. These disadvantages have to be met by an extensive program of education and state consultant services. Our first program of nursing education has been described elsewhere.[6] We are now working to develop a second state-wide educational program and to strengthen our consultation services, which are in heavy demand.

The first regulation to prevent and control outbreaks of diarrhea was incorporated into the New York State Sanitary Code in 1948.[7, 8] By 1952, it was generally believed that the problem of diarrhea in the newborn had been effectively surmounted. This complacency was abruptly shaken when the news of a large outbreak in a hospital in eastern New York State was made public in the summer of 1954. Results of state-wide inspections made after this outbreak showed that generally satisfactory progress had been made in the aspects reviewed, but that obvious deficiencies still existed in some hospital nurseries (TABLE 7). For these reasons, policies and procedures for enforcement were clarified and strengthened, and the regulation was completely rewritten. The opinion of physicians, hospital administrators, and public health personnel in upstate New York was clearly ranged on the side of making the rewritten regulations as specific and detailed as possible, and this was the course taken.

A full discussion of the regulation itself has been published elsewhere.[9] Only three important changes will be discussed here. Medical and nursing supervision of the nursery was not mentioned in the previous regulation.

TABLE 7

PERCENTAGE OF HOSPITAL NURSERIES WITH INADEQUATE FACILITIES OR FAULTY PRACTICES IN UPSTATE NEW YORK, 1948, 1949–51, AND 1954

	Year		
	*1948	†1949–1951	†1954
Inadequate facilities			
Less than 24 square feet of floor space per bassinet	66	32	10
Nurseries housing more than 12 infants	45	18	8
Faulty practices			
Same nurses caring for well and sick infants	14	3	8
Nurse-baby ratio greater than 1:12	33	14	7
Formula not terminally heated	58	9	5
Individual equipment for each infant not provided	53	8	5

* Questionnaire before State Regulations became effective.
† Hospital inspections.

Obviously this is not readily susceptible to government regulation. The suggestion that every hospital be required to designate a specific physician and nurse as responsible for the operations of the nursery for the newborn and for the preparation of written policies and procedures was received well, however, and is incorporated in the new regulation.

The reporting of diarrhea of the newborn has been a consistent problem. Unless cultures of a pathogenic microorganism are reported immediately, the diagnosis must necessarily be clinical. A fairly high proportion of normal newborn infants exhibit transitional soft, mushy stools during the first few days of life. Often an outbreak becomes evident only if someone takes the trouble to review the nursery records over a long period of time. The extreme importance of reporting outbreaks as soon as possible is very evident from our past experience (TABLE 4). An attempt to solve this apparent dilemma was made by incorporating in the regulation the following statement: "An immediate telephone report shall be made (by the hospital) to the local full-time health officer whenever: (1) an infant has one or more watery or explosive stools from any cause on two successive days; (2) any patient is admitted to the hospital with diarrhea (from any cause), the onset of which occurred when the patient was 28 days or less; and (3) the existence of diarrhea of the newborn is suspected."

Watery or explosive stools in newborn infants are pathological under any circumstances. When an infant under 28 days develops diarrhea, one should look into the nursery of the hospital where the infant was born. Neither of these two conditions indicates that an outbreak of diarrhea in a hospital nursery has occurred. They do alert the health officer, however, and permit him to undertake enough of an investigation to determine whether or not an outbreak exists. To date, experience with this new requirement has been satisfactory. An undue number of cases of diarrhea in infants under 28 days of age has not been reported and, in at least one instance, the reporting of a case led to the discovery of a minor outbreak of diarrhea that probably would not have been reported otherwise.

The value and importance of submitting *E. coli* isolated from stools to a laboratory equipped and staffed to do serogrouping and typing has been obvious to us in recent years. In several of the outbreaks reported within the last five years, satisfactory specimens were not obtained and, by the time the outbreak was reported, the suspects were receiving antibiotics, so that it was impossible to determine whether or not enteropathogenic *E. coli* were present. For these reasons, the Sanitary Code was revised to require the submission of specimens from suspected cases of diarrhea of the newborn to a local approved laboratory or to the Division of Laboratories and Research, Albany, N. Y. The Division of Laboratories and Research has prepared and distributed to approved laboratories two pools of *E. coli*-grouping sera for screening tests. Final serogrouping is done in the Central Laboratory.

Outbreaks of diarrhea on pediatric wards. Our present regulations cover only nurseries for the newborn. We have had experience with only one hospital outbreak that originated on the pediatric ward. The outbreak occurred early in 1955 and was initiated by the admission to the pediatric service of a two-month-old infant with diarrhea. Somewhat later in the course of events, *E. coli* O111:B4 was isolated from this infant's stools. The hospital is an excellent institution, but for many years it had cared for its premature infants on the same floor as its pediatric patients. We had actively discouraged this practice but had been unable to convince the hospital administration of its potential danger. We were told that the same nurses caring for pediatric patients would be prohibited from caring for the premature infants, thus eliminating the danger of contact spread, and that moving the nursery for the premature to the newborn service would be too expensive.

Diarrhea subsequently developed in two older infants on the pediatrics service and in two premature infants, one of whom died. *E. coli* O111:B4 was isolated from the stools of the surviving premature infant but not from the other infants, perhaps because of faulty technique. No cases of diarrhea occurred on the newborn service, located on another floor. In spite of hospital policy and state regulation, our investigation disclosed that nursing shortages had exerted an understandable pressure on the night shift. The same nurse, on several successive nights, had cared for the source of infection, the two older infants, and the two premature infants who developed diarrhea.

Shortage of trained nursery staff is probably the most critical factor affecting care of the newborn at the present time. Obviously, physical facilities of themselves cannot overcome such shortages. The presence of a premature unit adjacent to a pediatric ward, however, seems to demand too much forbearance in the human character, a point that can be generalized as the major reason for all requirements relative to nursery facilities. This small outbreak caused one preventable death. The hospital moved its premature unit without further urging. It is now adjacent to the nursery for the newborn, with which nursing services can be shared without the same dangers.

Summary

(1) Thirty-five reported hospital nursery outbreaks of diarrhea of the newborn in upstate New York during the nine-year period July 1, 1946 to June 30,

1955 have been reviewed. Outbreaks were most frequent in hospitals with large maternity services.

(2) The over-all case fatality rate for the 35 outbreaks was 11.8 per cent. Severity of outbreak appeared directly related to delay in reporting cases. Five outbreaks thought to be due to enteropathogenic *E. coli* had an over-all case fatality rate of 28 per cent.

(3) In every outbreak investigated, channels through which the microorganism could spread easily in the nursery situation were readily observable. These are discussed in some detail.

(4) Prevention and control of outbreaks hinges on elimination of all sources and channels of spread that can be eliminated easily and on the handling of each well baby at all times on the assumption that he is a potential source of infection.

(5) The New York State public health program of hospital nursery regulation has been described, emphasizing the decentralization of authority for inspection and enforcement, the need for a continuing state education and consultation program, and the importance of viewing regulatory authority as a positive effort to improve community health.

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ESCHERICHIA COLI AS A CAUSE OF DIARRHEA IN CALVES*

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Calf scours, white scours, calf diarrhea, and colibacillosis or Kälberruhr are terms used to designate a disease in calves that manifests itself by a marked catarrhal gastroenteritis and a fetid watery diarrhea that usually occurs 48 to 72 hours after the birth of the calf. The disease commonly terminates in death two to nine days after birth. Acute cases may terminate in death before diarrhea is evident. The recovery rate is highest in herds where morbidity is light and where calves have received colostrum. In herds where the infection has been established, the morbidity rate and the mortality rate rise markedly. Feeding colostrum under these circumstances tends to lower the incidence and mortality but apparently is incapable of stopping the spread of the disease. Essentially, calf scours is a disease of concentrated bovine populations and is seldom encountered under range conditions.

Isolation of the Etiological Agent

The etiological agent in calf scours is commonly regarded as *Escherichia coli.* The association of *E. coli* with calf scours was made at the turn of the last century by a number of European workers, including Jensen,[1] Nocard and Leclainche,[2] Joest,[3] and Titze and Weichel.[4] Later observations were made by Smith *et al.*[5-8] in the United States and by Lovell *et al.*[9-11] in Great Britain.

With the isolation of a causative organism, a number of important problems arose, many of which were subsequently solved. The answers to other problems remain somewhat nebulous in their interpretation by various workers. The present-day concepts of mixed infections, synergisms, animal viruses, and phage systems suggest innumerable possibilities for combination of etiological agents as the cause of enteritis of the newborn. One would be foolish, indeed, to disregard these newer concepts of infection and agent combination, for they may be just as important to the enteritis complex as weather, season, climate, sunshine, lack of vitamin A, overfeeding, synthetic diets, lack of colostrum, poor management, weak animals, and overconcentration of stock.

There still is much to be said in behalf of *E. coli*, however, for its part in this complex picture. Whether a primary or secondary factor, *E. coli* has been isolated from the blood stream and tissues of a high percentage of calves dying of calf diarrhea.

Although *E. coli* has been the most constant organism cultured from calves with scours, it has not been the only organism isolated. Titze and Weichel,[4] in a study of 104 animals with scours, isolated 210 strains of bacteria. Of these strains, there were 166 isolates of *E. coli*, 24 isolates of *Salmonella enteritidis*, two of paratyphoid B, and the rest miscellaneous bacteria. Lovell and Hughes[9] reported isolations from 69 cases. Infections with *E. coli* accounted for 37 cases, *Corynebacterium pyogenes* for 12, hemolytic coccobaccilli for 6, *Salmonella*

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typhimurium for 2, and *Pasteurella* for 1. Mixed infections accounted for 11 cases. Such organisms as *Staphylococcus aureus* and *Pseudomonas aeruginosa* were present in addition to certain of those encountered in pure infection. In an additional 31 cases the etiology was doubtful, indicating a nonbacterial cause or bacteria that could not be identified. Thorp *et al.*[12] isolated *E. coli* from the tissues of 15 animals dying of calf scours. Jones and Little[13] isolated *Vibrio jejuni* from the intestines of calves with diarrhea. Filterable agents have been described by Baker[14] and by Moll and Brandly[15] in relation to diarrhea of calves.

The Reproduction of Calf Scours in Susceptible Animals

Under natural conditions, calf scours appears almost spontaneously. Reproduction of the disease is simple in contaminated quarters. Because of this, much research has been done on organisms isolated from naturally infected animals. Experimental reproduction of the disease with strains isolated from infected animals, however, presents a different problem. Jensen[1] was able to produce a mild diarrhea by feeding cultures of organisms isolated from normal calves. A more severe reaction with subsequent death followed simultaneous administration of small amounts of creolin, methyl violet, or iodine trichloride in the milk. Jensen believed that the intestinal organisms simply acquired virulence if they contacted a damaged intestinal tissue. Further research, he added, proved that these organisms isolated from the blood and tissues of the above experimental animals were just as pathogenic as those isolated from calves dying from naturally occurring calf scours. Jensen cited J. Poels as being unable to duplicate the experiments with the chemical irritants and disagreed with Poels' theory that a specific strain of bacteria was necessary to cause the disease. Smith and Orcutt[6] were unable to cause the diarrhea by feeding cultures of *E. coli* to a 16-day-old calf that we now know was too old to be infected. Van Pelt,[16] citing unpublished data from the Storrs Experiment Station, Storrs, Conn., stated that cultures isolated from animals dying of calf scours were fed to calves taken from their dams at birth. The cultures were fed along with the colostrum and produced no ill effects. He reasoned that the protective action of the colostrum may have eliminated the effectiveness of the culture before it reached the small intestine. Since it was generally conceded that infection took place orally, Hagan[17] concluded that other agents were probably responsible for the primary infection and that invasion of the tissues by *E. coli* was secondary.

When an attempt was made to raise newborn calves on synthetic diets without colostrum, Aschaffenburg *et al.*[18] found that a high mortality from calf scours ensued. In contrast, he found that calves receiving colostrum for the first four days survived on this diet. Under similar conditions, Ingram *et al.*[19] showed that only 9 calves of a total of 103 survived an endemic of calf scours when deprived of colostrum. In the same experiment 118 calves of a total of 161 that received colostrum survived.

Another approach to the method of infection in the calf was offered by Williams *et al.*,[20] who attributed the infection by *E. coli* to intra-uterine exposure to the organism. He observed the occurrence of fetal diarrhea and instances of active dysentery at the time of birth. Such cases often recovered for a short period and then relapsed in 10 to 15 days. Many of the dams of calves that scoured suffered from metritis following calving or were afflicted with retained placenta. Williams criticized Jensen's work in that: (1) there was no evidence that the gastrointestinal tracts of the experimental calves were free from bacteria at the time of birth; (2) details or even a general outline of the feeding was lacking; and (3) most controls, as well as the inoculated calves, had diarrhea. The disease developed more slowly in the controls and was less frequently fatal but had the same general characteristics.

Transmission Experiments at Pennsylvania State University

The prevailing skepticism over the ability of isolated strains of *E. coli* to reproduce calf scours prompted transmission experiments with calves orginating from the Pennsylvania State University herd.

The history of the herd gave evidence that, in years past, calf scours had been a serious problem. In the year prior to these experiments, however, the Dairy Science Department had moved its herds into newly developed quarters and, for a time, calf scours ceased to be a problem. During this period the Veterinary Science Department also moved into new facilities and began transmission experiments.

Calves used in the experiments were males taken from their dams at birth, muzzled, and taken directly to the isolation unit. The unit into which a calf was placed was constructed with tile walls and floors and was thoroughly cleaned and disinfected before receiving the animal. The investigator entered the quarters through a vestibule where clean coveralls and boots were donned, and where hands were washed before entering the isolation unit. All calves received pasteurized milk from sterilized calf bottles and nipples. Milk was weighed and fed twice a day in amounts of two to three pounds per feeding, depending upon the size of the calf. The organism used in the experiments was a strain isolated from the tissues of a calf that had died from calf scours on a local farm. This strain* later proved to be an anaerogenic *E. coli* serotype O26:B6[21] identical with type 3 isolated from calves by Ørskov.[22] The organism was grown in veal infusion broth for six to seven days and then mixed with equal parts of additional veal infusion broth and incubated at 37° C. for two hours. Ten ml. of this culture was administered to the calf in the first milk it consumed. This six- to seven-day culture was made available for use upon the arrival of the calf at the laboratory by inoculating a tube of broth each day. Every week a new broth culture was started from the stock culture. Records were maintained on milk consumption, temperature, and the appearance of diarrhea throughout the experiment. Thirty-two calves deprived of colostrum and eight calves that received colostrum were used in this investigation. Four of the calves deprived of colostrum were used as controls and received no culture in the first feeding, while 22 calves deprived of colostrum

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* We wish to thank William H. Ewing of the Communicable Disease Center, Atlanta, Ga., for typing this culture.

TABLE 1

RESULTS OF EXPOSURE OF NEWBORN CALVES TO A PATHOGENIC STRAIN OF *E. COLI*

No. of calves	Exposure to *E. coli*	Received colostrum*	Became ill	Died	Recovered	No illness
22	Fed culture	No	20	14	6	2
7	Fed culture	Yes	3	1	2	4
4	None—control	No	0	0	—	4
6	Contact	No	6	6	0	0
1	Contact	Yes	0	0	—	1

* Received colostrum prior to being exposed to *E. coli.*

and seven that had received colostrum were given the infective organism. As shown in TABLE 1, 20 of the 22 calves not receiving colostrum became ill and developed a diarrhea. Fourteen of these died, six recovered, and two showed no illness. Of the seven calves receiving colostrum, three became ill and one of these died. Four showed no illness. The four control calves remained completely healthy throughout the experiment. In the other phase of the experiment, calves that had shown no illness during exposure to the feeding experiment were used as infected carrier contact animals. Six additional calves that had not received colostrum were placed in contact with one or more of these animals. The contact was made individually as the newborn calves were received at the laboratory by simply placing them in the same isolation unit with the carrier calves. All of the six animals thus exposed became ill and all died. One animal that received colostrum before exposure in this manner showed no illness. It should be mentioned that all of the six contact animals that died suffered from very acute cases of the disease. Two of them died of septicemia even before scouring became evident. A significant factor in these results was that calves being retained in the dairy herd that was the source of the experimental calves during this period had developed no cases of calf scours. This further emphasized the pathogenicity of the organisms fed in the experiment.

Another observation was that control calves that had received no colostrum showed no illness when exposed to *E. coli per os* several days after birth. In fact, one animal was exposed two days after birth and after four feedings of pasteurized milk. There was no evidence of illness.

None of the animals used in these experiments developed pneumonia. Even those that became ill and subsequently recovered showed no evidence of respiratory congestion or pneumonia.

It is recognized that these preliminary experiments left much to be learned. They did confirm the fact, however, that calves can be infected by oral administration of a specific strain of the organism. Furthermore, calves can be raised without colostrum and without calf scours if conditions are ideal. This seems to verify the concept that the organism is most effective when it is present in the first food the animal consumes. The data in TABLE 1 are somewhat misleading in that calves receiving no colostrum were exposed to the organism with the first feeding, whereas calves that had received colostrum were fed the organism at the next regular feeding.

Further Evidence that a Bacterial Agent is Involved

Those who would classify *E. coli* as simply a secondary factor in calf scours and who would favor the theory that a virus is the initiating factor will still agree that, in acute scours occurring clinically within 48 to 72 hours after birth, the infecting agent surely must be acquired at the time of parturition or shortly thereafter. With this in mind it is well to consider the effects of preventive treatments that could control bacterial but not viral infections.

During the months of December 1950 and January 1951, Bortree *et al.*[23] observed almost 100 per cent morbidity from calf scours in calves born in the Pennsylvania State University dairy herd. Beginning on January 21, 1951, each calf born was given a 500 mg. dose of Aureomycin within the first several hours after birth. Sixty-seven calves were born in the next seven months. Only one calf in this group died. That animal was weak and weighed only 35 lb. at birth. Forty of the 67 calves did not develop a diarrhea after the single oral treatment with Aureomycin. Twenty-one others required a second dose of Aureomycin within two to three days. The remaining four calves received additional treatment. When preventive treatment was discontinued, early in August 1951, 13 of 23 calves born in the ensuing 6 weeks developed scours.

Pearson[24] reported the effectiveness of oral Terramycin as a preventive treatment of calves for calf scours on six heavily infected farms. Twenty-one calves were given 0.5 gm. of Terramycin orally within the first 24 hours after birth. A similar dose was given in 12 to 24 hours, and no other precautions taken. Not one case of calf scours developed. Control calves were kept on two of the farms. All of these controls developed calf scours.

From these experiments it appears obvious that treatment of the intestinal tract of the newborn with a bacteriostatic agent on the day of birth and for not longer than the first two to three days after birth was sufficient to control severe endemics of calf scours.

If the primary infecting agent were a virus acquired at the time of birth and if it reached its peak of propagation within three days, it appears that there would still be ample time for secondary invasion by facultative pathogens even though their propagation had been temporarily inhibited by a bacteriostatic agent for the first 24 to 72 hours. In the experiments cited, however, the inhibition of excessive bacterial growth for 24 to 72 hours after birth effectively prevented the occurrence of calf scours. This is believed to be evidence of the control of a bacterial agent that was a primary etiological factor, rather than the control of a secondary invader.

Summary

Recognizing that other etiological factors have been shown to cause diarrhea in calves, the authors have attempted to present evidence from a review of the literature and from laboratory experiments to emphasize the importance of *E. coli* in the enteric conditions that occur within the first 72 hours after the birth of the calf.

Evidence presented in this paper, which strengthens the theory that *E. coli*

is a primary etiological agent in calf scours, may be summarized as follows:

(1) *E. coli* has been isolated more times than any other organism from calves dying of calf scours.

(2) Calf scours was effectively reproduced in susceptible calves under controlled conditions by feeding small amounts of a pathogenic strain of the organism in the first milk consumed after birth. Control calves in the laboratory and in the parent herd remained free of the disease.

(3) Control of intestinal bacteria at birth by bacteriostatic agents prevented the occurrence of calf scours in the endemics reported.

(4) The absence of pneumonia in animals dying from calf scours in the transmission experiments cited suggests that calf scours caused by *E. coli* is a different entity from the pneumoenteritis of viral etiology.

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THE ROLE OF *PARACOLOBACTRUM* AND *PROTEUS* IN INFANTILE DIARRHEA

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Probably the outstanding feature of the literature on the early bacteriology of infantile diarrhea is the consistent indication of the diverse etiology of this disease. The more recent recognition of the enteropathogenic *Escherichia coli* and its causal relationship to certain outbreaks of infantile diarrhea, adequately presented elsewhere in this monograph, has added still another bacterial species to the already long list of possible incitants of this clinical entity. The probability that one or more viral agents may also have to be included in the near future is indicated by other studies published in this monograph.

Time does not permit even a listing of the variety of bacterial species incriminated, at one time or another, as the cause of diarrhea in infants. It should be sufficient to note that members of such divergent genera as *Pseudomonas*, *Alcaligenes*, and *Staphylococcus*, as well as various types of enterococci, have been reported in sporadic or epidemic outbreaks. Prior to 1945, however, the agents most frequently reported, aside from the classical *Salmonella* and *Shigella*, were *Paracolobactrum* and *Proteus*.

The purpose of this presentation is to attempt to evaluate the role of these two groups in infantile diarrhea. The major obstacle encountered is the lack of any clear-cut demonstration of pathogenicity by these organisms for the gastrointestinal tract of the newborn. The alternative is to consider the evidence that has accumulated, almost since the original identification of these organisms, in the form of clinical, bacteriological, and epidemiological observations linking *Paracolobactrum* and *Proteus* to outbreaks of infantile diarrhea.

This is made difficult, especially in some of the early reports on *Paracolobactrum*, by the problems of classification. In addition, the knowledge that methods for the identification of the enteropathogenic strains of *E. coli* were not available makes it obvious that the investigations of the bacteriology of some of these reports could not have been complete and thus, to some extent, questions their validity. The latter problem is exemplified by the experience reported by Neter[1] in 1952 with stools obtained from two outbreaks of infantile diarrhea that had occurred in upper New York State in 1947. Previously, cultures of these stools had produced no evidence of the presence of any known pathogenic enteric bacteria, and attempts to isolate a virus, carried out in the laboratories of the New York State Health Department in Albany, N. Y., were unsuccessful. Fortunately, fecal samples had been frozen and, five years later, cultures of *E. coli*, O111 were obtained from five of the seven samples.

Almost from the time of their first recognition, so-called "paracolon" bacilli have been associated with infantile diarrhea, and although exact identification by the methods employed in early reports is impossible, there is little doubt that these refer to *Paracolobactrum*. There is also little doubt as to the authors' conclusions as to the etiologic roles of these agents in the disease. Thus, as far back as 1893, paracolon bacilli were described by Gilbert and Lion[2] in England as aberrant coliform organisms that fermented lactose slowly and were of clinical significance in infantile diarrhea but not in adult dysentery. Similar findings were reported by Morgan and Ledingham,[3] also in England, in 1909. Twenty years later, Fothergill[4] isolated 32 strains of late-lactose-fermenting coliform bacteria from infants suffering from acute diarrhea; these strains, he concluded, were "unagglutinable paratyphoid bacilli," and were of etiologic significance in infantile diarrhea.

More recently, attempts have been made to assess the pathogenicity of paracolon organisms by comparative studies of their occurrence in the gastrointestinal tracts of infants with diarrhea and in those of healthy infants. Comparative data by Neter and Clark[5] and Neter[6] indicated that in approximately 64 per cent of a group of 39 diarrheic infants from whom paracolon bacteria were isolated, no other potentially pathogenic enteric microorganisms were found, while the occurrence of paracolon bacilli in the stools of 50 healthy infants was reported to be 22 per cent. Neter concluded that these organisms may be the primary cause of diarrhea in small outbreaks in institutions, but that the significance of their presence in sporadic cases was difficult to determine. Similar conclusions were reached by Berger[7] in England concerning an organism he identified as *Bacterium wakefield* from cases of infantile diarrhea and which he could not detect in stools of healthy infants. Sevitt,[8] in Dublin, Republic of Ireland, reported isolation of paracolon bacteria from 26 per cent of 400 cases of diarrhea and enteritis and claimed that cultures of these organisms, when fed to kittens, produced an enterocolitis resembling diarrhea in infants.

While a few reports such as those by Stuart[9] and Plass,[10] show that anaerogenic strains of the genus *Providencia* have been involved in outbreaks of diarrhea in adults as well as in infants, other evidence has been presented that minimizes the role of *Paracolobactrum* even in outbreaks of infantile diarrhea where such organisms have been isolated. Thus, although Brown[11] in Australia isolated what was evidently an anaerogenic strain of *Providencia* from 13 cases of gastroenteritis, all of whom had demonstrable, specific agglutinins in their sera that were absent from sera of healthy infants, she indicated that the organisms were capable of causing only a mild gastroenteritis in infants. Similarly, Gauvreau[12] stated that the 25 infants in a group of 47 from which he isolated paracolon organisms during an outbreak of diarrhea in a Canadian foundling home were the least ill and had fewer stools, which convinced him of their unimportance.

Careful examination of the clinical, epidemiological, and bacteriological data included in these instances, and others not cited, confirm the conclusions of the various authors who mostly agree that *Paracolobactrum* may be the cause of outbreaks or small epidemics of infantile diarrhea, especially in institutions. Several extensive examinations of the sporadic occurrence of these organisms, however, have not been nearly as convincing. Buttiaux *et al.*[13] found anaerogenic paracolon strains in epidemic infantile diarrhea, but also reported these organisms present in 1.5 per cent of the stools of both healthy and diarrheic infants. Singer and Bar-Chay[14] isolated a strain of *Providencia* from 10 per cent of stools of 116 infants under 1 year of age who were suffering from diarrhea, but they also found these organisms in 3.25 per cent of 5400 stool specimens from diarrheic children of all ages. These authors were unable to come to a definite conclusion as to the pathogenicity of *Paracolobactrum*. Graber and Lincoln,[15] however, recently reported a thorough bacteriological study of the occurrence of *Providencia* paracolons in the stools of 341 infants under 1 year of age suffering from diarrhea in four Denver, Colo., hospital pediatric wards. In all instances, procedures for identifying all known bacterial agents of this disease were also performed. Paracolon organisms of all types were recovered, but the occurrence was approximately 3 per cent in 341 cases and included only 1 strain of *Providencia*. Furthermore, none of these could be incriminated as the etiological agent. Graber and Lincoln concluded that paracolon organisms comprised an unimportant part of the flora of diarrheic infants.

Various species of *Proteus* also have been associated with infantile diarrhea by many different investigators over a period of more than 50 years, beginning with Booker[16] in 1896, who stated that *Proteus vulgaris* was undoubtedly an incitant of "summer diarrhea" of infants but that it was seldom encountered in "mild diarrheas" of this age group. In 1914, Metchnikoff[17] concluded that *B. proteus* was the principal agent in diarrheal diseases of infants and claimed that monkeys fed infant fecal material developed diarrhea. Since that time numerous publications have appeared in which *Proteus* species have been cited as agents of infantile diarrhea and, during the last 15 years, most reports have usually dealt with 1 or 2 biotypes, especially *P. vulgaris* and *P. morganii*. In fact, several instances involving fatal infections with these two species have been reported in which attempts to prove the presence of other known agents were unsuccessful. Neter and Bender[18] and Neter and Farrar[19] between 1941 and 1943 reported a total of 18 cases of enterocolitis in infants due to either of these two *Proteus* species, 6 of which were fatal. Labrinacos *et al.*[20] cultured *P. vulgaris* from the stools of 20 infants involved in an outbreak of gastroenteritis in a foundling home in Athens, Greece. Five cases were fatal, and the authors concluded that *P. vulgaris* was the causative agent.

As with *Paracolobactrum*, this type of evidence seems to point to a definite capacity of these two *Proteus* species to act as incitants in small epidemics or outbreaks of infantile diarrhea. A third biotype, *P. rettgeri*, has been classified as a pathogen by Singer *et al.*,[21] who isolated it from 10.3 per cent of cases of diarrhea in infants and in only 0.89 per cent of healthy babies over a period from 1950 to 1954. This low incidence of *Proteus* in healthy infants was noted by Snyder[22] in 1940 during a study of the normal flora of the gastrointestinal tract of children between the ages of two weeks and one year. Such findings emphasize the possibility that these species may act as "true" pathogens in the intestinal tract of infants. In contrast to these observations, however, are the data of Graber and Lincoln[15] in which *Proteus* strains were encountered only 20 times in 607 stool cultures of diarrheic infants in Denver hospitals, while in a previous survey of 538 healthy and diarrheic infants in the same city, Graber and Dunlop[23] had noted that *Proteus* species were an insignificant part of the gastrointestinal flora.

Except for the evidence of Singer mentioned above, there is little or no evidence that either *Paracolobactrum* or *Proteus* organisms in the gastrointestinal tract of infants are "opportunists" causing individual or sporadic cases when innate or natural resistance is decreased. Assuming that the data presented here are sufficiently convincing of the capacity of some strains of these organisms to produce small epidemics or outbreaks of gastroenteritis in infants, the evidence available today indicates that certain strains of *Providencia*, and of *Proteus morganii*, *P. vulgaris*, and *P. rettgeri*, are "transient" flora capable of the role of "true" pathogens. Before such a conclusion can be reached, however, several factors inherent in the problem must be examined further. For example, considerable discrepancy appears in the reported occurrence of some of these organisms in healthy infants in surveys carried out in different parts of the world. This might be the result of the presence or absence of the carrier state about which nothing is known with reference to these organisms. Furthermore, as pointed out earlier, caution must be exercised in the interpretation of data on outbreaks in which complete examinations for all the known bacterial pathogens were not, or could not be, performed.

Aside from the epidemic form just considered, there is little evidence that either *Paracolobactrum* or *Proteus* is important as a causative agent of acute, infectious diarrhea in children under one year of age. The data of Graber and Lincoln demonstrate that organisms of each group were isolated in only 3.5 and 6 per cent, respectively, of 341 cases. This work included examinations for all known bacterial pathogens. It is perhaps even more interesting that none was demonstrable in 242 cases, or approximately 71 per cent (TABLE 1). No tests for the presence of viral agents were performed, but the need for information concerning the possibility of viruses alone, or in combination with bacteria, as possible etiologic factors in this disease is obvious. Other studies of the bacterial flora of the feces of nonepidemic cases of the disease have shown results similar to those of Graber and Lincoln.

In summary, certain species of *Paracolobactrum*, particularly certain anaerogenic strains of *Providencia*, and of *Proteus vulgaris*, *P. morganii* and *P. rettgeri* have been associated frequently with outbreaks or epidemics of infantile diarrhea, especially in hospitals and institutions housing children of less than one year of age. None of these agents is encountered regularly in nonepidemic or sporadic cases, and their incidence in the feces of healthy infants is also quite low. These facts suggest that the role of these agents in epidemics in which they are found as etiologic agents is that of "true" pathogens. It was also

TABLE 1
THE INCIDENCE OF POTENTIALLY PATHOGENIC ENTEROBACTERIA IN 341 CASES OF INFANTILE DIARRHEA

Total cases	*Salmonella, Shigella*		*E. coli* serotypes		Paracolon bacteria		*Proteus*		None	
	No.	%	No.	%	No.	%	No.	%	No.	%
341	17	4.98	50	14.6	12	3.5	20	5.8	242	71

pointed out that complete examination of the bacterial flora of nonepidemic cases of diarrhea in children under one year of age failed to determine the cause of a majority of these cases.

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THE BACTERIAL HEMAGGLUTINATION TEST FOR THE DEMONSTRATION OF ANTIBODIES TO ENTEROBACTERIACEAE*

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INTRODUCTION

The laboratory diagnosis of enteric infections due to gram-negative bacilli, with the notable exception of typhoid fever, is based largely on the recovery of the pathogen from the patient. The serological diagnosis on the basis of the demonstration of an increased titer of bacterial agglutinins is not carried out routinely. In part, this is due to the fact that in infections caused by *Salmonella*, *Shigella*, and *Escherichia* a large battery of antigens representing various serogroups or serotypes is required; and, to some extent, the enterobacterial agglutination test is not sufficiently sensitive, particularly in young infants, to detect low levels of antibodies. Studies of the enterobacterial hemagglutination test have revealed, as will be shown in this paper, that this method overcomes to a large extent the above-mentioned shortcomings of the conventional agglutination test. Observations on the monovalent and polyvalent enterobacterial hemagglutination tests and the hemolytic modification of these tests are recorded here, and the potential usefulness of these methods for the diagnosis and epidemiological investigation of enterobacterial diarrheal disease is documented.

MATERIALS AND METHODS

Antigens. Crude antigens of *Salmonella*, *Shigella*, and *Escherichia* were prepared as follows:

The strains were grown on brain veal agar (Difco) in Kolle flasks at 37° C. for 18 to 20 hours. The resulting growth was harvested in 25 ml. of phosphate buffer (pH 7.3; Difco) or in unbuffered, physiological saline solution. The suspension was then heated in boiling water for one hour, and the supernate was obtained by centrifugation in the refrigerated centrifuge. This supernate was then ready for use in the hemagglutination test. The heated suspension was used as antigen in the conventional bacterial agglutination test. Treatment of the unheated supernate (saline) with NaOH for the activation of the antigen was carried out according to the method of Macpherson *et al.*[1]

Purified lipopolysaccharides were prepared by the phenol/water extraction method of Westphal and his associates, followed by fractionation with organic solvents and ultracentrifugation.[2, 3] Some of these lipopolysaccharides were freed of nucleic acids, others were not. Several *Escherichia coli* lipopolysaccharides were made available by the Difco Laboratories, Detroit, Mich., through the kindness of William H. Schoenlein. These purified antigens were

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dissolved in phosphate buffer for use in the unheated or heated (1 hour at 100° C.) form or in physiological saline solution for subsequent NaOH treatment, as mentioned above.

Sonic-treated bacterial suspensions were kindly prepared in the magnetostrictive oscillator (Raytheon Manufacturing Company, Waltham, Mass., Model DF-101) by W. S. Hammond of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

Hemagglutination and hemolysis tests. The enterobacterial hemagglutination test was carried out as follows:

Red blood cells of sheep or man (blood group O) were washed three times in phosphate buffer in order to remove serum that inhibits the adsorption of enterobacterial antigens on erythrocytes.[4-6] To the packed red blood cells was added the respective crude or purified antigen in suitable dilution. The final concentration of red blood cells was 2.5 per cent. The mixture, after thorough shaking, was incubated in a water bath at 37° C. for 30 minutes. In some experiments this treatment was carried out also at other temperatures, as mentioned later. The treated (modified) erythrocyte suspension was then washed three times in phosphate buffer in order to remove excess antigen, which may interfere with hemagglutination or hemolysis. The suspension of modified red blood cells (volume 0.2 ml.) was then added to antiserum in serial dilutions (0.2 ml.) obtained from immunized animals or procured from patients. The mixtures were incubated in a water bath at 37° C. for half an hour and then centrifuged at approximately 1000 rpm for 1 to 2 minutes. The resulting agglutination of the red blood cells was read grossly. Mention should be made of the fact that in a strongly positive test the erythrocytes are agglutinated in a single solid clump. In the hemolytic modification of this hemagglutination test, guinea pig (Carworth Farms; dilution 1:20) or human complement (volume 0.1 ml.) was added to the modified red blood cells and antiserum. Hemolysis was read after incubation at 37° C. for 30 minutes. In a few experiments the incubation period was 2 hours, and the resulting hemolysis was read after centrifugation by inspection of the supernate. Minimal but definitive hemagglutination or hemolysis were taken as end points in titration tests.

The polyvalent enterobacterial hemagglutination test and its hemolytic modification were performed by treating red blood cells with a mixture of either crude or purified lipopolysaccharide antigens.

Hemagglutinin absorption tests. Two different methods were used in absorption experiments. In one, either the antiserum (volume 0.2 ml.) in suitable dilution was mixed with 0.1 ml. of the purified enterobacterial antigen in serial dilutions or, alternatively, a standard amount of the antigen was added to the serum in serial dilutions. The mixtures were incubated in a water bath at 37° C. for 15 minutes. Appropriately modified red blood cells were then added, and the hemagglutination test was carried out as described above. In the second type of absorption experiment, packed, modified red blood cells were used for absorption of the antiserum. The mixture was incubated at 37° C. for 15 minutes. The absorption was repeated as necessary, and the supernate was then titrated as described above. Appropriate controls (heterologous antigen, phosphate buffer, or untreated red blood cells) were included.

Bacterial agglutination test. In the bacterial agglutination test, antiserum in serial dilutions (volume 0.25 ml.) was mixed with equal amounts of the heated bacterial suspension. Incubation was carried out at 50° C., and the resulting agglutination was read grossly after 1 hour and 18 hours, respectively.

All serum specimens were inactivated for 30 minutes at 56° C. prior to antibody titration. Unless used immediately after procurement, they were stored in the deep freeze. All comparative hemagglutination and bacterial agglutination tests were carried out with the identical serum dilutions. Whenever more than one serum specimen from a patient was titrated, the tests were done on the same day with identical indicator systems.

Antigen titration. Titration of the enterobacterial antigens was carried out by means of the hemagglutination or hemolysis inhibition tests as follows: serial dilutions of the antigen (volume 0.1 ml.) were mixed with 3 to 4 minimal hemagglutinating or hemolyzing units of the corresponding antiserum. The mixtures were kept in a water bath at 37° C. for 15 minutes. Suitably modified red blood cells were than added, and the hemagglutination test (or its hemolytic modification in the presence of complement) was carried out as described above.

RESULTS

Basic Aspects of the Enterobacterial Hemagglutination Test

Studies of crude and purified enterobacterial antigens. In the preliminary study of the *Escherichia coli* hemagglutination test, unheated bacterial suspensions were employed, and only equivocal results were obtained. It was not until heated suspensions were used that clear-cut modification of red blood cells was effected.[7, 8] Analysis of the effects of heat on the erythrocyte-modifying capacity of the bacterial suspension revealed two independent results: (1) that heat liberates large amounts of antigen from the bacterial cells into solution, and (2) that heat enhances materially the erythrocyte-modifying capacity of the soluble crude antigens. These conclusions are based on the results of repeated experiments, a typical one being summarized in TABLE 1. Both the suspension and the supernate were titrated for antibody-neutralizing and erythrocyte-modifying capacities. It will be noted that the antibody-neutral-

TABLE 1

EFFECTS OF HEAT ON ANTIBODY-NEUTRALIZING AND ERYTHROCYTE-MODIFYING CAPACITIES OF *E. COLI* ANTIGENS

Tests	Titers of			
	Unheated		Heated	
	E. coli O111 antigens			
	Suspension	Supernate	Suspension	Supernate
Antibody neutralization	1:400	1:200	1:3200	1:200
Erythrocyte modification	<1:50	negative (1:1)	1:3200	1:160

Table 2

The Antibody-Neutralizing and Erythrocyte-Modifying Capacities of Supernates of *E. coli* O111 Suspension Before and After Exposure to Sonic Energy

Tests	Titers of					
	"Sonic-treated"			Control		
	Supernates					
	Unheated	Heated	NaOH-treated	Unheated	Heated	NaOH-treated
Antibody neutralization	1:6400	1:1600	1:3200	1:800	1:400	1:400
Erythrocyte modification	negative (1:1)	1:2000	1:4000	negative (1:1)	1:200	1:100

izing capacity of the heated suspension is far greater than that of the unheated suspension, whereas no such difference exists between unheated and heated supernates. Both the unheated suspension and the unheated supernate failed to modify red blood cells. After being heated, however, they readily accomplished modification. In fact, the heated preparations were from 60 to 160 times as effective as red blood cell-modifying antigens than the unheated preparations. Similar results were obtained with sonically treated suspensions, as summarized in TABLE 2. In this particular experiment, the effect of NaOH on the antigen was also studied. Both heat and NaOH treatment enhanced substantially the erythrocyte-modifying capacity of the supernates without causing a corresponding increase in antibody-neutralizing ability. Studies to be reported elsewhere in detail[9] and already briefly summarized[10] revealed that heat and sodium hydroxide treatment have the same effects on purified enterobacterial lipopolysaccharides as they do on the crude soluble antigen; namely, they enhance the erythrocyte-modifying but not the antibody-neutralizing potencies.

Purified enterobacterial lipopolysaccharides, even without treatment with heat or NaOH, may modify red blood cells although these treatments have

Table 3

Minimal Amounts (μg/ml.) of *Salmonella* Lipopolysaccharides for Modification of Erythrocytes

Groups of *Salmonella* lipopolysaccharides	*Salmonella* lipopolysaccharides		
	Untreated	Heated	NaOH-treated
S. paratyphi A (A)	100	25	10
S. paratyphi B (B)	200	65	1.25
S. thompson (C_1)	>1000	12	2.5
S. newport (C_2)	250	6	5
S. gallinarum (D)	6	1.5	0.6
S. anatum (E_1)	>1000	12	0.6
S. newington (E_2)	12	3	0.6

invariably enhanced the red blood cell-modifying capacity of these compounds. Recently, purified lipopolysaccharides were prepared from strains of *Salmonella* representative of the more common serogroups. The erythrocyte-modifying capacity of the unheated, heated, and NaOH-treated antigens was determined by means of the hemagglutination test and homologous *Salmonella* antisera; the latter were kindly supplied by Ivan Saphra of the New York *Salmonella* Center, New York, N. Y. The results of these experiments are summarized in TABLE 3. It is evident that there are remarkable differences in the minimal amounts of these untreated lipopolysaccharides sufficient for red blood cell modification. Furthermore, both heat and NaOH treatment substantially enhance this capacity, although to different degrees with different antigens. It remains to be determined whether these results are characteristic of the respective lipopolysaccharides or are due to the particular process of preparation of the individual strains used.

Studies of modification of red blood cells. It was previously shown[11] that in the absence of electrolytes, erythrocytes are not modified by crude *E. coli* antigens. Further studies have revealed that electrolytes are needed also for the adsorption of purified enterobacterial lipopolysaccharides, inasmuch as modification does not take place when the treatment is carried in 5 per cent glucose or 5 per cent sucrose solution, but is readily effected when isotonic solutions of NaCl, KCl, or sodium citrate are used. It is interesting to mention that isotonic $CaCl_2$ solution does not permit adsorption of the purified antigens by erythrocytes. This observation has been made repeatedly, provided that $CaCl_2$ solution is used (1) as diluent for antigen, and (2) as washing and suspending fluid for erythrocytes, prior to, during, and after treatment with the antigens. On the other hand, the hemagglutination test itself is carried out in buffer solution. The reason for the failure of $CaCl_2$ to allow red blood cell modification remains obscure and requires further investigation.

Myron A. Leon, of Saint Luke's Hospital, Cleveland, Ohio, called our attention to his observation of the temperature dependence of the erythrocyte-modification phenomenon (personal communication). Our own studies have revealed that, indeed, modification with crude and purified enterobacterial lipopolysaccharides takes place only to a minimal or questionable degree at 2° C. Room temperature permits better modification, although not to the same extent as do 37° C. and 50° C. These observations eventually may aid in the elucidation of the precise nature of the modification reaction.

It has been shown previously that red blood cells are capable of adsorbing several antigens, either simultaneously or consecutively.[8, 12, 13] Modification of red blood cells by six different *E. coli* lipopolysaccharide antigens has been accomplished, as shown in TABLE 4. It is seen that erythrocytes modified with either one or six lipopolysaccharides are equally good tools for the titration of the respective antibodies. Identical results were obtained with red blood cells treated with several *Salmonella* and *Shigella* antigens. The potential usefulness of this polyvalent hemagglutination test for the detection of any homologous antibodies in the serum of patients was first pointed out by Neter and Walker.[14] Data obtained with the polyvalent hemagglutination test are recorded in TABLES 4, 7, 11, and 13.

Table 4

Monovalent and Polyvalent *E. coli* Hemolysis Tests

E. coli antisera (serogroups)	Hemolysis of sheep red blood cells modified by *E. coli* lipopolysaccharides							
	O8	O25	O26	O55	O86	O111	O127	Mixture of all antigens
	Titers (reciprocal)							
O8	1000	—	—	—	—	—	—	1000
O25	—	1000	—	—	—	—	—	1000
O26	300	—	2700	—	—	—	—	2700
O55	—	—	—	5000	—	—	300	5000
O111	—	—	—	—	—	2700	—	2700
O127	—	—	—	—	—	—	2000	2000

Symbol — = less than 200.

Enterobacterial hemagglutination versus hemolysis. It was shown previously that modified sheep red blood cells are readily lysed in the presence of rabbit antiserum and guinea pig complement.[8] In contrast, no clear-cut hemolysis could be observed when modified human red blood cells were used in the same system, although sheep and human erythrocytes were agglutinated by the antiserum to the same degree. The question arose as to whether lysis of modified human red blood cells could be affected in the presence of antibodies of human origin and of either guinea pig or human complement. An answer to this question was obtained by the use of a human serum devoid of antibodies but exhibiting complement function. This serum from a patient with agammaglobulinemia was kindly made available by James F. Holland of the Roswell

Table 5

Agglutination and Lysis of *Shigella flexneri* Modified Erythrocytes by Serum of Patient with *S. flexneri* Dysentery

Serum dilutions	Agglutination of		Lysis of			
	Human O	Sheep	Human O		Sheep	
	Cells		Cells in presence of			
			Guniea pig	Human	Guinea pig	Human
			Complement			
1/50	4	4	—	—	4	4
1/100	3	3	—	—	4	3
1/200	3	3	—	—	2	2
1/400	2	2	—	—	1	1
1/800	1	1	—	—	—	—
1/1600	—	—	—	—	—	—
0	—	—	—	—	—	—

Symbols: 0 = No agglutination or lysis; 1 to 4 = Various degrees of agglutination or lysis.

TABLE 6
COMPARATIVE EFFICACY OF *E. COLI* (O111) LIPOPOLYSACCHARIDE
HEMAGGLUTINATION AND HEMOLYSIS TESTS

Sheep erythrocytes modified with lipopolysaccharide in amounts (μg/ml)	Titers (reciprocal) of *E. coli* antiserum causing	
	Hemagglutination	Hemolysis
500	12,800	25,600
125	6400	25,600
31	3200	12,800
8	1600	12,800
2	800	6400
0.5	100	3200

Park Memorial Institute, Buffalo, N. Y. The results of this study are presented in TABLE 5. It is evident that modified human erythrocytes are not lysed in the presence of either guinea pig or human complement, although both complements readily effect lysis of modified sheep red blood cells, and the antiserum causes agglutination of both sheep and human erythrocytes.

In view of the observation of Leon[15] to the effect that the optimal temperature for lysis using human complement is from 30° C. to 32° C., the above experiments were repeated at this temperature. Again, no lysis occurred with modified human red blood cells, whereas correspondingly treated sheep red blood cells were readily lysed. The reason for the refractoriness of human erythrocytes to lysis in this system remains to be elucidated. It is clear that human red blood cells do not lend themselves as tools in the enterobacterial hemolysis test.

It was previously shown that the hemolytic modification of the *E. coli* hemagglutination test proved to be considerably more sensitive than the hemagglutination test when antisera obtained from rabbits, guinea pig complement, and sheep red blood cells were used.[8] Further studies have revealed that, as shown in TABLE 6, the hemolysis test is markedly more sensitive than the hemagglutination test with weakly modified erythrocytes, but only slightly more sensitive with strongly modified red blood cells.

APPLICATIONS OF THE ENTEROBACTERIAL HEMAGGLUTINATION TEST

The E. coli *hemagglutinin response of infants with diarrheal disease due to enteropathogenic* E. coli. Previous studies on the antibody response of volunteers to the ingestion of *E. coli* O55 revealed that the titer of homologous antibodies was from 5 to 20 times higher with the hemagglutination method than in the conventional bacterial agglutination test.[16] The superiority of the hemagglutination method was revealed also in the study of the antibody response of infants suffering from diarrheal disease due to *E. coli* O26.[17] Since that time, observations have been made on other infants with sporadic diarrheal disease caused by enteropathogenic *E. coli*. The data are summarized in TABLE 7. It can be seen that rises in the titer of specific antibodies could be demonstrated by means of the hemagglutination method but not with the bacterial agglutination test. Furthermore, the table reveals that red blood

TABLE 7

E. coli HEMAGGLUTININ RESPONSE OF INFANTS WITH *E. coli* DIARRHEAL DISEASE

Patient	Age	*E. coli*	Date of blood specimens	Titers (reciprocal) of			
				E. coli hemagglutinins*			Bacterial agglutinins
				O111	O26	O111 or O26†	
E. J.	3 months	111	1-8-55	—	—	—	—
			2-3-55	80	—	80	—
S. C.	4 months	111	2-4-55	—	—	—	—
			3-4-55	80	—	80	—
J. B.	10 months	26	2-16-55	—	—	—	—
			2-22-55	—	80	80	—
D. H.	3 months	26	2-16-55	—	—	—	—
			2-24-55	—	6	6	—
			3-10-55	—	12	12	—
			3-30-55	—	24	24	—

Symbol: — = less than 5 or 6, respectively.

* O cells modified with culture supernate in dilution of 1:2.

† O cells modified with both antigens.

cells modified with two different *E. coli* antigens were agglutinated by the sera of the patients as well as those treated with the single homologous antigen only. In these particular tests crude *E. coli* antigens were used. It can be seen from the data presented in TABLE 8 that purified *E. coli* O111 antigen was as suitable as the crude antigen. With both antigens an increase greater than eightfold was obtained in this seven-weeks-old infant. Attention should be called to the fact that the relatively low levels of hemagglutinins demonstrable in young infants necessitate the use of lower serum dilutions and/or of higher concentrations of the antigen. One must keep in mind also that interpretation of the results on a single blood specimen must be based on the occurrence of hemagglutinins in the particular age group of the population. In a study by the authors[18] the distribution of *E. coli* hemagglutinins in various age groups was determined. It was found that these hemagglutinins were absent or present in low titer only in the blood of infants, and that the majority of older children and adults had such antibodies in higher titers. In the newborn, passively transferred hemagglutinins may be present, although in lower titers than in the maternal blood. It is obvious that passively acquired antibodies

TABLE 8

E. coli HEMAGGLUTININ RESPONSE OF INFANT WITH E. COLI O111 DIARRHEAL DISEASE

Date of serum specimens	*E. coli* hemagglutinin titers (reciprocal) for O cells modified with		
	E. coli O111 culture supernate	*E. coli* O111 lipopolysaccharide (20 μg./ml.)	*E. coli* O26 culture supernate
2-4-55	<10	<10	<10
2-8-55	<10	<10	<10
3-5-55	80	80	<10

TABLE 9

RELATIVE EFFICACY OF *SALMONELLA* HEMAGGLUTINATION AND AGGLUTINATION (CENTRIFUGE MODIFICATION) TESTS

Serum dilutions	Antibodies in serum of patient with *Salmonella enteritidis* gastroenteritis against							
	S. enteritidis				*E. coli* (patient's strain)			
	Hemagglutinins		Agglutinins		Hemagglutinins		Agglutinins	
	a	b	a	b	a	b	a	b
1:20	3	4	1	3	1	4	1	3
1:40	3	4	—	2	1	4	—	3
1:80	2	4	—	1	—	3	—	2
1:160	2	4	—	—	—	2	—	1
1:320	1	3	—	—	—	1	—	—
1:640	—	2	—	—	—	—	—	—
1:1280	—	1	—	—	—	—	—	—
1:2560	—	1	—	—	—	—	—	—
1:5120	—	1	—	—	—	—	—	—
1:10.240	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—

Symbols: a = before centrifugation; b = after centrifugation; 1 to 4 = various degrees of hemagglutination or agglutination; — = no hemagglutination or agglutination.

decrease in titer with age, and that an increase in the titer of these antibodies is due to the active formation of hemagglutinins. It is gratifying to the authors to learn that Stulberg and Zuelzer have been able to confirm, in principle, our results with the hemagglutination method and have shown the formation of *E. coli* O127 hemagglutinins in some of their patients.[19]

The Salmonella *and* Shigella *hemagglutination tests.* Studies from this laboratory have revealed that the hemagglutination test is a suitable method for the demonstration of the antibody response of children suffering from diarrheal disease due to *Shigella sonnei* and *Salmonella*.[14, 20] Recently, Braun, Seeliger, and Wagner[21] reported that the bacterial agglutination test can be made more sensitive if the mixture of antigen and patient's serum is centrifuged after an appropriate period of incubation. For this reason it became of interest to compare the sensitivities of the hemagglutination with that of the centrifugation modification of the agglutination test. Furthermore, it was considered important to determine the effects of *Salmonella* or *Shigella* infection on the heterologous antibody response, particularly on that against the patient's

TABLE 10

HOMOLOGOUS AND HETEROLOGOUS ENTEROBACTERIAL HEMAGGLUTININS IN SERUM OF PATIENT WITH *SALMONELLA TYPHIMURIUM* ENTERITIS

Titers (reciprocal) of hemagglutinins

S. typhimurium	*S. choleraesuis*	*S. münchen-oregon*	*E. coli* O111	*E. coli* O55	*S. flexneri*	*S. sonnei*
2650	—	40	—	—	—	—

Symbol: — = less than 20.

TABLE 11

HOMOLOGOUS AND HETEROLOGOUS HEMAGGLUTININ RESPONSE OF PATIENT WITH *SHIGELLA FLEXNERI* INFECTION

Dates of blood specimens	Titers (reciprocal) of hemagglutinins against							
	Patient's *S. flexneri*	Patient's *E. coli*	*S. flexneri*	*E. coli* O111	*E. coli* O55	*S. cholerae-suis*	*S. typhimurium*	All antigens
10-3-55	40	80	—	10	—	10	10	80
10-11-55	2560	160	20	80	40	80	80	2560

Symbol: — = less than 10.

own strain(s) of *E. coli*. The possibility exists that the lesion in the intestinal tract may make possible the entrance into the circulation of bacterial antigen from the nonpathogenic bacterial flora of the intestinal tract, resulting in antibody formation.

For these reasons, the study of the homologous and heterologous hemagglutinin responses of patients with salmonellosis and shigellosis was undertaken. TABLE 9 gives the data obtained on a patient with *Salmonella enteriditis* gastroenteritis. It can be seen that (1) centrifugation of the mixture of bacterial suspension and patient's serum enhances the resulting agglutination, (2) centrifugation also markedly increases the agglutination of modified red blood cells after incubation with the patient's serum, (3) the titer of *Salmonella* hemagglutinins is 64 times higher than that of the corresponding bacterial agglutinins, and (4) the titer of *S. enteriditis* hemagglutinins is 16 times higher than that of *E. coli* hemagglutinins. In numerous other cases, too, the hemagglutinin titers were invariably higher than those of bacterial agglutinins.

In many of our patients the titers of *Salmonella* or *Shigella* hemagglutinins were far higher than those of hemagglutinins acting on heterologous antigens. A typical example is summarized in TABLE 10, which reveals that *S. typhi-*

TABLE 12

HEMAGGLUTININ RESPONSE TO PATIENTS' OWN STRAINS OF *SHIGELLA FLEXNERI* AND *ESCHERICHIA COLI*

Dates of blood specimens	Titers (reciprocal) of			
	Hemagglutinins		Agglutinins	
	Against homologous			
	S. flexneri	*E. coli*	*S. flexneri*	*E. coli*
Sibling 1				
10-3-55	40	160	—	—
10-7-55	160	320	—	—
10-11-55	2560	640	40	—
Sibling 2				
10-7-55	80	320	—	—
10-15-55	2560	320	640	—

Symbol: — = less than 20.

TABLE 13

SEROLOGICAL EVIDENCE OF DOUBLE INFECTION WITH *SHIGELLA FLEXNERI* AND *SALMONELLA CHOLERAESUIS*

Dates of blood specimens	Titers (reciprocal) of hemagglutinins							
	S. flexneri	*S. sonnei*	*E. coli* O111	*E. coli* O55	*S. choleraesuis*	*S. typhimurium*	*S. san diego*	All antigens
10-25-55	40	—	—	—	320	20	20	320
10-31-55	640	20	—	—	1280	40	20	640

Symbol: — = less than 20.

ABSORPTION OF HEMAGGLUTININS

Serum	Hemagglutinins	
	S. flexneri	*S. choleraesuis*
Unabsorbed	800	400
Absorbed with *S. flexneri*	—	200
Absorbed with *S. choleraesuis*	800	—

Symbol: — = less than 50.

murium antibodies were present in a titer exceeding those of heterologous antibodies by 64 to 128 times.

The changes of homologous and heterologous antibody titers during an attack of shigellosis or salmonellosis were then investigated. The results obtained on a patient with *Shigella flexneri* dysentery are summarized in TABLE 11. This table shows that the patient's *Shigella* hemagglutinins increased sixty-fourfold in eight days, whereas only eightfold increases occurred in antibody titers against several heterologous antigens. It is noteworthy that in absolute terms, too, the titer of hemagglutinins against the pathogen was far higher than the titers against heterologous antigens. It is interesting to point out that in a number of other patients, rises in the titers of heterologous antibodies did not

TABLE 14

SEROLOGICAL EVIDENCE OF CO-PRIMARY SHIGELLOSIS IN FAMILY

Members of family	Titers (reciprocal) of *Shigella sonnei* hemagglutinins on		
	8-18-55	8-25-55	9-3-55
Sibling 1	10	160	80
Sibling 2	—	160	160
Sibling 3	—	160	80
Sibling 4	10	640	640
Sibling 5	20	320	320
Sibling 6	10	100	n.d.
Sibling 7	10	320	n.d.
Father	10	40	n.d.
Mother	40	40	n.d.

Symbol: — = less than 10; n.d. = not done.

TABLE 15

ENTEROBACTERIAL HEMAGGLUTINATION TEST FOR SEROLOGICAL EPIDEMIOLOGY OF *SHIGELLA SONNEI* DYSENTERY

Family members	Titers (reciprocal) of hemagglutinins	
	S. sonnei	All heterologous antigens*
Patient	1280	160
Mother	from 40 to 320	80 (no increase)
Sibling 1	160	80
Sibling 2	640	80
Sibling 3	from 80 to 320	no increase
Sibling 4	1280	160
Sibling 5	1280	160
Sibling 6	1280	160

* Two *E. coli*, two *S. flexneri*, five *Salmonella*.

take place. That this is not always due to the absence of pre-existing antibodies is evident from the data presented in TABLE 12. Two siblings with *Shigella flexneri* dysentery were studied, and it was found that both patients developed *S. flexneri* hemagglutinins in identically high titer. Although antibodies against the patients' own strains of *E. coli* were present at the onset of the infection, only one patient showed a rise in the titer of homologous *E. coli* hemagglutinins.

The question arises as to whether hemagglutinins against heterologous enterobacteriaceae develop because of the presence of common antigens. This is unlikely, since rises against several rather unrelated enteric bacteria were observed. Furthermore, absorption experiments clearly showed that the hemagglutinins were removed only by homologous and not by heterologous antigens. In these experiments both absorption methods were used; namely, addition of the supernate of a heated suspension to the serum of the patient and treatment of serum with modified red blood cells. The reason why some patients do not produce heterologous hemagglutinins while others do remains to be elucidated. It is possible that the presence of development of heterlogous antibodies in moderately high titers (1:40 to 1:320) may be related to the age of the patient, previous contact with these or related antigens, and/or nonspecific stimulation (anamnestic reaction).

The enterobacterial hemagglutination test appears to be a valuable tool for the serological diagnosis of enterobacterial infections. For example, studies on a patient with bacteriologically proved *Salmonella choleraesuis* infection suggests that this patient also was infected with *Shigella flexneri*. This conclusion is based on the results of hemagglutination and absorption studies, as shown in TABLE 13.

Also, the enterobacterial hemagglutination test has proved to be helpful in supplying serological evidence of co-primary infection in a family. An illustrative example is presented in TABLE 14. It can be seen from this table that all seven siblings developed *Shigella sonnei* hemagglutinins within one week. During this time there was no change in the titer of these antibodies in the mother. The children suffered from severe to very mild shigellosis.

TABLE 16

ENTEROBACTERIAL HEMAGGLUTINATION TEST FOR SEROLOGICAL EPIDEMIOLOGY OF SALMONELLOSIS

Family members	Titers (reciprocal) of hemagglutinins			
	S. typhimurium	*S. sonnei*	*E. coli* O111	*E. coli* O55
Patient	10,240	—	40	80
Sibling 1	640	—	—	—
Sibling 2	640	20	20	20
Sibling 3	320			—
Sibling 4	—	n.d.	n.d.	n.d.
Sibling 5	10	n.d.	n.d.	n.d.
Sibling 6	10	n.d.	n.d.	n.d.
Mother	10	n.d.	n.d.	n.d.

Symbol: — = less than 20; n.d. = not done.

Finally, the enterobacterial hemagglutination test promises to be a valuable tool for serological epidemiology of enterobacterial infections. TABLES 15 and 16 summarize data obtained in the investigation of two large families. Study of six siblings and the mother of a child with *Shigella sonnei* dysentery revealed that all members except one had *Shigella sonnei* hemagglutinins in high titer (1:640 or above) or showed a fourfold to eightfold increase, and that the titers of all heterologous hemagglutinins were considerably lower or showed no increase. These data suggest that most members of the family may have been infected with this microorganism at one time or another. Similar results were obtained in the investigation of the family of a patient with *Salmonella typhimurium* infection, as may be seen from the data presented in TABLE 16. It should be pointed out that in this family three siblings had *S. typhimurium* hemagglutinins in relatively high titer, whereas the other three siblings and the mother did not. All members of the family except the patient were well. It is reasonable to assume that the children with high *S. typhimurium* antibody titers may have been infected previously with *Salmonella* or an antigenically related microorganism. In view of the fact that in sporadic cases of salmonellosis, and not rarely of shigellosis, the source of the infection remains obscure, the enterobacterial hemagglutination test may well offer the opportunity to obtain more adequate information on subclinical infection and possible sources.

DISCUSSION

In the foregoing study, evidence has been presented that the enterobacterial hemagglutination method is more sensitive than the conventional bacterial agglutination test for the demonstration of the antibody response of patients with enteric infection. The development of the polyvalent enterobacterial hemagglutination test was made possible by the fact that several antigens, both crude and purified, can be simultaneously adsorbed on red blood cells. The suspension of these modified erythrocytes is a useful tool for the demonstration of any homologous hemagglutinins (*cf.* TABLES 4, 7, 11, 13). It appears likely that this polyvalent enterobacterial hemagglutination test may be useful for the screening of sera of patients and contacts. For appropriate interpretation it will be necessary to determine the titers of the respective hemagglutinins in the population unless one examines at least two sera, one taken at the onset of the illness and the other one or two weeks later, and demonstrates a marked increase in the titer of these antibodies. The polyvalent hemagglutination test makes unnecessary the use of a battery of different antigens in the conventional agglutination test, aside from the fact that it is more sensitive. It may be particularly valuable as a screening test. The enterobacterial hemagglutination method also may become a useful tool for studies of the epidemiology of enteric infections. Several examples have been presented.

For a long time the role of certain paracolon and *Proteus* bacilli as etiological agents of enteric infection has remained obscure. It is conceivable that determination of the hemagglutinin response of patients with diarrheal disease may supply suggestive evidence for or against this assumption. The antibody response of human volunteers to the ingestion of enteropathogenic *E. coli* but not to that of a "normal" *E. coli* has supplied evidence of the etiological role of these microorganisms in infantile diarrhea.[22, 23] The development of specific *E. coli* hemagglutinins by infants suffering from diarrheal disease has been shown in the present communication.

Finally, it may be useful to explore the possibility of the utilization of the enterobacterial hemagglutination test for the demonstration of coproantibodies and of the hemagglutination-inhibition test for the determination of the presence of enterobacterial antigen in the feces of patients with diarrheal disease. If the latter method were feasible, it can be anticipated that an early etiological diagnosis may be made possible.

Summary and Conclusions

Studies on basic aspects and applications of the enterobacterial (*E. coli*, *Salmonella*, *Shigella*) hemagglutination test were carried out, and the following results were obtained:

(1) Both crude and purified enterobacterial antigens can be adsorbed on red blood cells. These modified red blood cells can be used for the determination and titration of the corresponding antibodies.

(2) Absorption of purified lipopolysaccharides does not take place in 5 per cent glucose or 5 per cent sucrose solutions, nor in isotonic $CaCl_2$ solution, but is effected readily in isotonic saline, potassium chloride, and sodium citrate solutions.

(3) Modified human red blood cells—in contrast to sheep erythrocytes—are not lysed in the presence of homologous bacterial antibodies and human or guinea pig complement, either at 30 or 37° C.

(4) Several antigens can be absorbed simultaneously, thus making possible a polyvalent enterobacterial hemagglutination test.

(5) The enterobacterial hemagglutination test has been shown to be considerably more sensitive than the conventional bacterial agglutination test and its centrifugation modification.

(6) Specific antibody responses of patients with *E. coli*, *Salmonella*, and *Shigella* diarrheal disease have been recorded.

(7) Some patients show increases of a moderate degree in the hemagglutinin titers against heterologous enteric bacilli, whereas others do not.

(8) The potential usefulness of the hemagglutination method for the determination of the coprimary infections and for serological epidemiology of enteric diseases has been documented.

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Discussion of the Paper

Horace L. Hodes and Helen D. Zepp (*Department of Pediatrics, Mount Sinai Hospital, New York, N. Y.*): We have in fact developed a screening test for the detection of enteropathogenic *E. coli* in stools. This method depends on the extraction of type-specific antigen from samples of fecal matter. These antigens give a sharp precipitate with homologous serum. The test is so sensitive that it yields positive results when only minute quantities of serum are used (capillary tube method).

HEMAGGLUTINATION WITH RED CELLS SENSITIZED WITH ANTIGENS OF ENTEROPATHOGENIC *ESCHERICHIA COLI*

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Sensitization of red cells with the envelope antigen of the Enterobacteriaceae for use in hemagglutination tests was demonstrated by Spaun[1] in 1952, using the Vi antigen from a boiled extract of *Salmonella typhi*. Neter,[2] the same year, adsorbed what he believed to be the O antigen of *Escherichia coli* O111 onto red cells used to detect hemagglutinins in infants with infantile diarrhea due to this agent. Thus far there have been no published reports to indicate that the same thing can be done with the B component, an envelope antigen present in all the enteropathogenic *E. coli* and responsible for O inagglutinability of these strains in OB serum. Wheeler[3] has suggested a method of using the B antigen very similar to that employed by Spaun. This method is based on the assumption that the B antigen is more rapidly adsorbed to red cell surfaces than the O antigen. Stulberg[4] utilized essentially the same principle in his work, but somewhat modified the procedure used by Wheeler. Results obtained with specific sera from animals immunized with whole organisms indicated the presence of B antigen on the red cells. O antigen was also adsorbed, however, if the red cells were exposed for a longer time, suggesting that both antigens were present in these extracts. The carbohydrate nature of the B antigen of the enteropathogenic serotypes, in contrast to the lipid-protein-carbohydrate complex of the O antigen, suggested a solubility differential in water favoring elution or extraction of the former from the bacterial cells as well as selective adsorption to normal red cells which is characteristic of many carbohydrates.

The following is a preliminary report of some attempts to demonstrate the B antigen-antibody system of *E. coli* O127:B8 by hemagglutination using red cells sensitized esssentially with an aqueous extract of the bacteria.

Materials and methods. The antigen used in the hemagglutination test was a suspension of group O human red cells sensitized with an aqueous extract of the organisms. Erythrocytes sensitized with O antigen according to the method described by Neter[2] were employed for comparison. Extract was prepared by suspending 4 gm. of wet weight *E. coli* O127:B8 from cultures on blood agar base in 15 ml. of deionized water that were incubated in a water bath at 37° C. for one-half hour. The suspensions were then centrifuged for 1 hour at 10° C. and the cells discarded. Isotonicity was restored with NaCl, and the extract was passed through a Selas filter. A 1 per cent suspension of human group O red blood cells was sensitized by incubation in various dilutions of this extract in a water bath at 37° C. for one-half hour.

Results. Red cells sensitized with several dilutions of extract were quite readily agglutinated in specific immune OB rabbit serum previously prepared by four intravenous injections of whole bacterial cells. Optimal sensitizing dilutions were determined on several extracts. Typical results are presented in TABLE 1. Usually the optimal dilution for sensitization was 1:5. Preliminary chemical analysis with anthrone indicated that considerable carbohydrate was present

TABLE 1

HEMAGGLUTININ TITRATIONS OF OB IMMUNE SERUM WITH RED CELLS SENSITIZED WITH DILUTIONS OF EXTRACT

Extract	Dilutions of serum								
	2	4	8	16	32	64	128	256	512
1:5	s	s	s	s	s	s	s	3	—
1:10	s	s	s	s	s	3	—	—	—
1:30	s	—	—						
1:60	—	—	—						

S = solid agglutination of red blood cells.

TABLE 2

HEMAGGLUTININ TITRATION OF UNABSORBED AND EXTRACT-ABSORBED OB SERUM

Serum	Absorbed	Antigen	Serum dilutions					
			4	16	64	256	1024	2048
OB	—	Ext.	s	s	s	s	s	0
OB	—	Neter	s	s	s	s	s	4
OB	Ext.	Ext.	s	s	0	0	0	0
OB	Ext.	Neter	s	s	s	s	s	2

in the extracts and was reduced by approximately 25 per cent by the red cell sensitization.

TABLE 2 illustrates hemagglutination titrations of another OB antiserum with red cells sensitized with the extract, and with O-sensitized red cells, before and after adsorption with the former.

The original titer for extract-sensitized cells was 1:1024, and dropped to 1:16 following adsorption. Titers of the same serum tested with O-sensitized cells prepared according to the Neter method were approximately the same before and after three adsorptions with extract sensitized cells. Further adsorption of this serum tended to lower titers for both O- and extract-sensitized cells. Hemagglutinins for extract-treated cells, however, were completely removed from one serum having an original titer of 1:256 for such cells without affecting the original O titer.

In order to confirm the presence of antibody with a specificity other than O as indicated by absorption experiments, an attempt was made to absorb O hemagglutinins. Data in TABLE 3 indicate that absorption of OB serum with red cells sensitized with O antigen by the Neter method resulted in approximately equal reduction of the original titers for both kinds of cells.

Results of an attempt to demonstrate specificity of the extract by agglutination titration of the serum with appropriate bacterial suspensions before and after absorption with the extract-sensitized cells are presented in TABLE 4 and are in essential agreement with results obtained by hemagglutination. The agglutinin titer with living organisms inagglutinable by O antibody was reduced from 1280 to 80 after absorption with extract sensitized cells, but only a one-tube drop occurred in the O agglutinin titer after this absorption.

TABLE 3
HEMAGGLUTININ TITRATION OF OB IMMUNE SERUM BEFORE AND AFTER ABSORPTION WITH O ANTIGEN

Serum	Absorbed with	Antigen	Serum dilutions					
			4	16	64	256	1024	2048
OB	—	Net.	s	s	s	s	s	4
OB	—	Ext.	s	s	s	s	s	—
OB	Net O	Net.	s	s	s	—	—	—
OB	Net O	Ext.	s	s	s	s	—	—

TABLE 4
BACTERIAL AGGLUTININ TITRATION OF UNABSORBED AND EXTRACT-ABSORBED OB SERUM

Serum	Absorbed with	Antigen	Serum dilutions					
			10	40	80	320	1280	5120
OB	—	OB	4	4	4	4	3	—
OB	—	O	4	4	4	4	3	2
OB	Ext.	OB	4	4	4	—	—	—
OB	Ext.	O	4	4	4	4	3	—

A further difference between extract antigen and O antigen was observed in the quantity and the rate of production of antibodies to each produced in rabbit serum following injection of OB cells, as seen in FIGURE 1. O agglutinins appeared earlier and reached peak titer earlier than B agglutinins. The production of hemagglutinins for extract-sensitized cells practically paralleled that of B agglutinins.

Finally, both the aqueous extract and the Neter O extract were tested against an OB rabbit antiserum in an agar double diffusion system. A wide band of precipitate occurred in the extract-antiserum mixture midway between the antigen and serum discs in approximately 24 hours. Subsequently it increased in width. Another faint band appeared several days later very close to the extract-antigen disc. About the same time a similar faint band appeared near the disc containing the O antigen, which suggested that this band was produced by O antigen antibody precipitation, and that this antigen might also be present in the aqueous extract. After four or five days a band of precipitation corresponding to the position of the earlier wide band found with extract antigen appeared in the O extract-antiserum mixture. Although this latter band was very faint and narrow its presence could indicate a small amount of extract antigen in the O preparation.

Discussion. These data do not afford proof for the specificity of the aqueous antigen. The carbohydrate nature of the antigen, the differential water solubility, the selective absorption onto normal red cells, and the specificity and antigenic capacity in rabbits as compared to the O antigen all seem, however, to warrant such an assumption. Undoubtedly the failure to separate the so-called B antibody by absorption of O antibody in antiserum with either bacteria

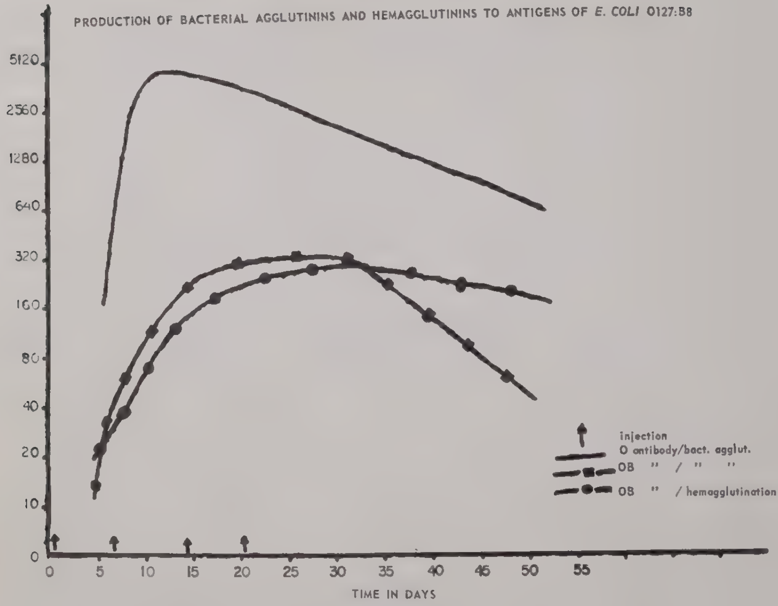

FIGURE 1

or with O sensitized red cells is the greatest objection to conclusions as to specificity. Experience to date, however, suggests that this may not indicate a lack of specificity other than O in extract antigen, but rather that antibody common to both extract (B antigen) and O antigen is present in OB antiserum. If so, it seems probable that the common specificity results from structural similarities in the carbohydrate portion of the O complex and in the extract antigen. This is indicated further by the fact that the most successful absorptions occurred with early antiserum obtained from animals that had received only one injection of bacteria, suggesting that more prolonged immunizations may, in effect, reduce specificity by either stimulating production of more common antibody, or by the previously observed fact that many carbohydrate antigens induce maximal antibody production only by rather small, optimal dosage. This possibility is indicated again by the observation that production of both agglutinins for the living bacteria and hemagglutinins to extract sensitized red cells is not particularly enhanced by further injections of large amounts of antigen. Data not presented here seem to confirm this impression that perhaps careful study of the immunization procedures with reference to specific B antibody may be more productive. It is also interesting that the titers for the hemagglutinins obtained in these experiments parallel closely the values obtained for agglutinins (anti B) to the living bacteria, in contrast to the findings of Wheeler, Stulberg, and Neter, in which hemagglutinin titers of OB serum usually were significantly higher than either type of bacterial agglutinins.

Assuming the B specificity for extract antigen, it is obvious that the procedure of extracting or eluting the antigen with water has certain advantages in time and mildness in contrast to previous procedures employed by other workers. If, as indicated by the agar diffusion data, the aqueous extract contains a small amount of O antigen, it is apparently of no consequence in the hemagglutination reaction, the O antigen probably being present in quantities insufficient to sensitize red cells adequately. Results of the agar diffusion experiments also provide some confirmation of Neter's claim that the antigen in his extract is primarily O antigen.

Summary. An aqueous extract of *E. coli* O127:B8 was prepared that was capable of sensitizing human red cells for the demonstration of hemagglutinins in OB rabbit antiserum. Preliminary observations indicate the probability that this extract contains primarily the B antigen.

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THE ROLE OF CLOSTRIDIA IN DIARRHEAL DISEASES OF MAN

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The clostridia are so widely distributed in nature and are such common and constant inhabitants of the human intestinal tract that, since the earliest days of clinical bacteriology, they have been isolated and frequently implicated in a great variety of disease processes. Unhappily, it is one thing to recover an organism from some pathological lesion, and it is another to show that this organism is an active agent in the genesis of that lesion. At once it must be emphasized that while the role of the clostridia in wound infections and, in particular, in gas gangrene was soon established, the evidence for their being involved in any sort of enteric infection in man (however strongly this might be suspected) was much less satisfactory, and has, in fact, been obtained beyond reasonable doubt only within the past decade. These more recent data, however, make it clear that we now have to deal, not with a single new disease, but with a number of distinct syndromes, separable both clinically and bacteriologically.

The problem, then, is a difficult one, and is in no way simplified by the fact that the infecting organisms are extremely closely related. All belong to the *Clostridium perfringens* group.

In a publication such as this, there is neither the space nor the necessity to review the fantastically complex toxicology of the pathogenic clostridia, but I should like to remind you that many of these organisms elaborate more than one toxin and that, in consequence, it has been found possible to divide certain species into a number of distinct types or varieties, depending either on the different toxins or different combinations of toxins they produce. In this way no less than six types of *Cl. perfringens* have been recognized (TABLE 1).

Nor is this classification to be regarded merely as a tour de force on the part of the immunologist. It is also of the greatest epidemiological significance (TABLE 2).

Two things in particular stand out from this tabulation. First, that *Cl. perfringens* might well be regarded as primarily an intestinal pathogen; second, that it is even more important in veterinary than in human pathology. Fortunately for me, the animal infections are to be the subject of a separate paper by E. M. Baldwin, but there still remain three types—A, D, and F—each of which is now known to be capable of producing more or less severe diarrheal disease in man. For a number of reasons, some of which will only become apparent later, I propose to discuss these organisms separately.

Cl. perfringens *Type A*

It is probably safe to say that, so far as man is concerned, *Cl. perfringens* Type A is by far the most important of the pathogenic clostridia or, indeed, of the pathogenic anaerobes. Classically, this organism is associated with various necrotizing wound infections, of which gas gangrene is the best known and most

TABLE 1
TOXIGENIC TYPES OF *CL. PERFRINGENS*

Type	Toxin											
	α	β	γ	δ	ϵ	η	θ	ι	κ	λ	μ	ν
A	+++	−	−	−	−	(+)	++	−	++	−	+	+
B	+	+++	+	+	++	−	+	−	−	+++	+	+
C	+	+++	+	++	−	−	+	−	+	−	−	+
D	+	−	−	−	+++	−	+	−	+	+	+	+
E	+	−	−	−	−	−	+	++	+	+	−	+
F	+	+	+	−	−	−	−	−	−	−	−	+

TABLE 2
PATHOGENICITY OF *CL. PERFRINGENS*

Cl. perfringens	Disease
Type A	Gas gangrene and food poisoning in man Enterotoxemia of calves
Type B	Lamb dysentery of sheep Enterotoxemia of foals
Type C	"Struck" in sheep Hemorrhagic enteritis of calves
Type D	Infectious enterotoxemia of sheep Pulpy kidney disease of lambs Two cases of enteric infection in man
Type E	Enterotoxemia of calves (?)
Type F	Enteritis necroticans of man

severe. Its involvement in diarrheal disease, though long suspected,[1-5] was not certainly established until 1953, when Hobbs and her colleagues in England[6] published an exhaustive study of a number of outbreaks of food poisoning in which there could be no doubt that *Cl. perfringens* was the causal organism. These observations have since been amply confirmed.[7-10]

Apart from their relative mildness, cases of *perfringens* food poisoning differ in no way from those due to many other intestinal pathogens. Nearly all the outbreaks have been associated with the ingestion of meat dishes, and the usual story is that the food has been prepared and cooked 24 hours or more before consumption, allowed to cool slowly at room temperature or in the refrigerator, and then heated up immediately prior to serving. *Cl. perfringens* has invariably been isolated from the suspected foods (in which it has always been present in enormous numbers, and frequently in pure culture) and also from a very high proportion of the stools of those stricken. Moreover, in volunteers, the ingestion of pure cultures of the organisms has produced symptoms of the disease.

Although recognizable as belonging to Type A, these food-poisoning strains of *Cl. perfringens* differ in several respects from the classical ones—in their toxigenic capacity (which is low), in their antigenic structure, and in their marked ability to produce heat-resistant spores. It is fortunate, of course, that these differences exist; in their absence the etiological role of *Cl. perfringens* Type A could not have been so certainly established, for this organism is an almost constant inhabitant of the human intestine.

The natural habitat of the food-poisoning strains has not, however, been fully determined. The organisms are very rarely found in normal human stools, but are of more frequent occurrence in the feces of pigs, cattle, rats, and mice. In sewage they seem to be present in from 60 to 100 per cent of all samples examined. More interesting perhaps is their very high incidence in blowflies, an observation that clearly indicates one mode of infection.

The importance of this form of food poisoning cannot as yet be accurately assessed, but is undoubtedly much greater than is generally appreciated. According to a report by the British Ministry of Health in 1954,[10] *Cl. perfringens* accounted for 5 per cent of all known outbreaks of food poisoning in Great Britain in 1951–1952. This is certainly a minimal figure for, at that time, the results of Hobb's investigations had not been published, and anaerobic cultures were not being generally undertaken in the routine bacteriological examination of such cases. This is a point worthy of the strongest emphasis, more especially in view of the fact that in a large number of outbreaks of food poisoning no pathogenic bacteria can be identified by use of the usual laboratory procedures.

So far we have been considering what is almost certainly an exogenous infection. There still remains the problem of endogenous infection by strains of *Cl. perfringens* normally present in the gut. Inevitably the evidence for such a syndrome is much more difficult to obtain, but it seems to me that the studies of Howie and his colleagues[11, 12] in Glasgow, Scotland, do suggest that it may occur. Their work, which is admittedly of a preliminary nature, has been concerned with the diarrhea that occasionally follows major surgery on the gastrointestinal tract. This condition has received a considerable amount of attention in recent years, but so far no very satisfactory explanation of its origin has been forthcoming.[13-16] Briefly, Howie and his collaborators report that in a series of cases of postgastrectomy diarrhea, typical *Cl. perfringens* Type A was found in unusually large numbers, not only in the stools, where it might be expected, but also in the gastric remnant, where it does not normally occur.[17] Moreover, in 8 of the 15 cases studied, free alpha toxin was identified in the stomach contents. More mysterious was the finding that filtrates of the loose stools neutralize considerable amounts of alpha toxin. Whether this effect was due to antitoxin remains to be determined, but Howie informs me that it can only in part be attributed to the presence of nonspecific inhibitors. Only minute traces of antitoxin were found in the blood of these patients.

There, for the moment, the matter rests. No very definite conclusions can or should be drawn from these observations, but the results of the more extensive studies now underway by Howie and his colleagues in Glasgow will be awaited with interest. In particular, we should like to know more about the conditions that predispose to the development of pathogenicity in intestinal strains of *Cl. perfringens.*

Cl. perfringens *Type D*

Although of considerable interest to bacteriologists, human infections with *Cl. perfringens* Type D are probably too rare to merit more than brief notice here. This anaerobe, long recognized as the cause of various enterotoxemic diseases of sheep, was not known to be pathogenic for man or even to occur in the human intestine. This year, however, reports of two cases have been published—one of intestinal obstruction and one of severe diarrhea-complicating amoebic colitis—in which there is every reason to believe that Type D strains played a significant part.[18, 19] Not only was the organism found in large numbers in the stools or intestinal contents of these cases but, in one, the major toxin, epsilon toxin, was identified in the strangulated loop of the small bowel while, in the other, epsilon antitoxin appeared to a significant titer in the patient's serum.

Epidemiologically these cases are probably to be regarded as little more than oddities, yet they do serve to underline one important and quite possibly essential feature of many intestinal infections with *Cl. perfringens*, namely the influence of predisposing factors. It is well-known that agents that lower the Eh of the tissues help to determine the onset of gas gangrene in wound infections, and it seems clear that analogous though not necessarily identical conditions may operate in enteric disease. Thus in both of these cases of Type D infection the gut was already abnormal, in one case from strangulation and in the other from amoebic infestation. Similarly, in the endogenous Type A infections it might be argued that the preliminary trauma of major surgery had in some way altered the tissue reactivity of the bowel. In this connection it is interesting to recall that in animal experiments with *Cl. perfringens* type D, Bennetts[20] failed to produce infection by feeding fully toxigenic strains to sheep unless he had more or less paralyzed the small intestine by the administration of morphine.

The whole problem is a fascinating one and should readily lend itself to experimental investigation.

Cl. perfringens *Type F*

Cl. perfringens Type F, the cause of enteritis necroticans, is unique among the organisms we have been discussing in that to date it has been found only as a pathogen of man. Taxonomically, it appears to be closely related to *Cl. perfringens* Type B (the cause of lamb dysentery), from which it differs in various minor points of toxicology and in its ability to produce extremely heat-resistant spores.[21, 22] Nevertheless, enteritis necroticans might well be considered as the human form of lamb dysentery.

This infection, the "Darmbrand" of the German writers, first appeared in northwest Germany toward the end of World War II, rapidly assumed epidemic proportions, and then, after a few years, mysteriously vanished.[23-27] A small number of cases from other parts of the world, including the United States, have been reported in the last few years, but in none of these can the specific bacteriological diagnosis be regarded as satisfactory. There is, however, some evidence that a similar syndrome occurred in the same area after World War

I, and it has also been claimed that the outbreak originated on the Eastern Front during the final retreat of the German armies; but all of this is uncertain and we are still in ignorance of the true origin of the disease.

Cl. perfringens Type F occurs in large numbers in the stools of typical cases and can also be identified in the gangrenous areas of the gut.[22] Antibodies to the beta toxin, the most important toxin produced by Type F strains, have been identified in convalescent sera.[2] Feeding *Cl. perfringens* orally to guinea pigs has been unsuccessful, but the introduction of young cultures directly into the lumen of the small intestine has produced a condition indistinguishable from that occurring in man.[22]

As its name indicates, enteritis necroticans is a severe and frequently gangrenous infection of the small intestine. The disease is acute and has a high fatality rate, in some series of cases, as high as 40 per cent. Apart from the unusual degree of shock and toxemia, the signs and symptoms are those of any similar lesion of the small bowel and need not be detailed here. Infection apparently occurs from eating contaminated foods, of which various preserved meat and fish products have been by far the commonest sources.[22, 25, 28] The infectious process is presumably the same as that occurring with the heat-resistant Type A strains. The food is first heated for some hours with a view to preservation; this kills off all other organisms except the Type F strains, which rapidly multiply in the slowly cooling material. The predisposing causes of this condition, if any, are not known, but it has been suggested with some reason that the severe malnutrition widespread in Germany after both World Wars may have been of importance.

There is little to add to this brief review. I believe it not at all unlikely that the other "animal types" of *Cl. perfringens* may yet be found to be causing enteric disease in man, although it is improbable that they could be of much importance in this respect. It is also worth bearing in mind that there are a number of reports in the literature that suggest that, in addition to *Cl. perringens*, various other species of spore-bearing bacilli, both aerobic and anaerobic, may be involved in this type of infection. The fact is that much of this work is so recent and, I must add, so little known that we are not yet in a position to assess the relative significance of such organisms in diarrheal disease. I feel sure, however, that this significance is greater than is generally believed.

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CLOSTRIDIA IN DIARRHEAL DISEASES OF ANIMALS

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Clostridium perfringens is the only known species of the genus *Clostridium* that can be directly implicated as the cause of diarrhea in animals. The term enterotoxemia is used to designate the diseases caused by the various types of *Cl. perfringens*. Diarrhea is frequently a symptom of these diseases. Type C enterotoxemia of infant calves and lambs and Type D enterotoxemia of sheep are the only disease entities recognized in the United States. They will be subject to detailed discussion under separate headings following the literature review.

Review of Literature

Cl. perfringens has been divided into six types, A, B, C, D, E. and F, on the basis of the toxins produced. From these six types, 15 soluble, antigenic toxic fractions have been identified and are designated by letters of the Greek alphabet. Oakley and Warrack[1] and Van Heyningen[2] have indicated that each of the six types of *Cl. perfringens* produce one or more of the antigenic fractions. Generally, one of the fractions is produced in quantity and is termed the major toxin, whereas other fractions are termed minor.

The toxins of *Cl. perfringens* Type A, of which alpha is the major toxin, are responsible for the production of gas gangrene in man and, occasionally, in domestic animals. Macrae, Murray, and Grant[3] have described an enterotoxemia in young suckling calves characterized by sudden onset, diarrhea, and early mortality. From cultures of fecal material, they isolated Type A. Rose and Graham,[4] in 1936, described an enterotoxemia jaundice of sheep and calves that they attributed to *Cl. perfringens* Type A.

Cl. perfringens Type B produces beta and epsilon antigens as the major toxins and is responsible for lamb dysentery, a highly fatal disease occurring during the first four days of life. In Great Britain, Gaiger and Dalling (1921), who first attributed the disease to a combination of the colon *Bacillus* and a member of the Welch group, later concluded that only the Welch organism was the etiological factor. This they called the "L.D. Bacillus" and showed that it differed essentially from the classical *Bacillus welchii*. Wilson (1931) called this organism Type B in his classification. Hepple[5] described a necrotic enterotoxemia in a calf that he attributed to Type B. No report could be found of the isolation of Type B in the United States.

The major toxic antigen of Type C is beta toxin, a lethal and necrotizing toxin. McEwen and Roberts,[6] in 1931, described a disease of sheep from Romney Marsh in England that they called "struck." McEwen isolated a member of the Welch group that was designated *Bacillus paludis*. Later, *B.*

paludis became Type C in the Wilsdon classification of *Clostridium welchii.* Struck is an enterotoxemialike disease of mature sheep that has not been reported in this county. In an earlier publication Griner and Bracken[7] described an acute hemorrhagic enteritis in young calves, and Griner and Johnson[8] reported a similar disease in infant lambs, both caused by Type C. Heller,[9] in 1933, gave an account of an acute hemorrhagic enteritis in young shed-born lambs in California. Filtrates of intestinal contents were lethal to mice, but the toxic constituent was not identified. A recent report by Fiedl and Gibson[10] incriminated *Cl. perfringens* Type C as the etiological agent of an enterotoxemia of young suckling pigs. The disease was characterized by lassitude, inappetence, weakness, and diarrhea, with death occurring approximately 12 hours after the onset of symptoms.

Cl. perfringens Type D is the cause of enterotoxemia in sheep and goats. It is generally distributed throughout the world. The primary toxic fraction of Type D is epsilon toxin, a lethal and necrotizing toxin that needs to be activated by trypsin and other proteolytic enzymes. The minor toxins of Type D are alpha and theta.

Bosworth[11] has described a strain of *Cl. perfringens* isolated from a calf showing symptoms of an acute enterotoxemia. This strain has been classified as Type E, and it produced alpha toxin and a new toxin that he designated as iota.

Cl. perfringens Type F is characterized by the ability of its spores to withstand boiling for one to four hours. Alpha, beta, gamma, and nu toxins are produced by this type. In Europe, Type F is believed to produce enteritis necroticans,[12] an acute toxemia fatal in man.

Hemorrhagic Enterotoxemia (Type C Enterotoxemia)

Distribution. Hemorrhagic enterotoxemia of calves and lambs has been observed and confirmed in numerous areas in Colorado since 1951. Other states in which the disease has been confirmed in suckling calves are Montana, Nebraska, and Wyoming.

Unconfirmed reports have been received from 10 states—California, Idaho, Illinois, Indiana, Iowa, Ohio, Oregon, South Dakota, Texas, and Utah. Type C toxins of *Cl. perfringens* have been found in newborn lambs in Colorado, Montana, and Wyoming.

It is believed that unfavorably cold, wet, windy weather at calving time has some influence on the severity of outbreaks of the disease.

Species. Hemorrhagic enterotoxemia has been most frequently observed in beef breeds of cattle. In a few instances, however, it has occurred in dairy breeds. No record has been kept on the breeds of lambs in which the disease has occurred.

Symptomatology. Field observations generally indicate that calves dying from hemorrhagic enterotoxemia are large, well-formed, and apparently vigorous animals. Stockmen who have experienced losses have confirmed these observations. They report that the disease most frequently attacks healthy, vigorous bull calves and that small, weak calves are rarely lost. It is generally agreed among cattlemen that calves dying of hemorrhagic enterotoxemia are from dams that produce an abundance of milk. There is no agreement that the disease occurs with greater frequency in calves from mature cows than in calves from heifers. Individual cows are reported to have lost their calves for two or three consecutive years.

Many stockmen are of the opinion that the incidence of the disease is greater than mortality figures indicate. They believe that calves develop subacute cases characterized by diarrhea from which they recover but remain unthrifty. This opinion is supported by serological studies in which the dams carried no antitoxin titer while their calves had titers ranging up to 16 units of Type C antitoxin. It is further confirmed by the observation that in vaccinated herds the incidence of diarrhea and unthriftiness is markedly reduced below "normal."

The symptoms displayed by individual calves vary with the degree of intoxication. In many instances calves are found dead. Early in the disease, infected calves become listless and ceased nursing. These symptoms may be noticeable 12 to 24 hours prior to the onset of severe symptoms. Later they display evidence of acute colic such as uneasiness, straining, and kicking at the abdomen. Hemorrhagic diarrhea may or may not be observed, depending largely upon the severity and duration of illness. Calves become prostrate, develop opisthotonos, and tetanic and toxic spasms, followed by death. The course of clinical cases of the disease varies from 2 to 24 hours. Temperatures generally remain near normal but, terminally, become subnormal.

Few clinical symptoms other than early death are observed in lambs. Shivering, bleating, and other evidence indicative of chilling or colic are observed. Few lambs show evidence of a hemorrhagic diarrhea. The lambs become weak, prostrated, and die.

Necropsy findings. Lesions appear to be consistently more severe in calves and are primarily hemorrhagic in character. The most marked pathological change is an extensive necrotic hemorrhagic enteritis of the jejunum and ileum. The lumen of the intestine contains considerable blood and necrotic mucous membrane. The intestinal contents are deep red, and portions of the large intestine may be hemorrhagic. Petechial or ecchymotic hemorrhages are consistently observed on the epicardium and thymus and, inconsistently, in the diaphragm, abomasum, and parietal pleura.

The lesions in lambs are spotted along the intestinal mucosa. Ecchymotic subserous hemorrhages may occur on the ansa spiralis, cecum, and small intestine. The peritoneal cavity contains small quantities of a serosanguineous fluid. A mild fibrinous peritonitis covering the inflamed intestine may be observed. Serohemorrhagic lymphadenitis of the mesenteric lymph nodes is frequently found. Hemorrhages are occasionally observed in the thymus and diaphragm. The pericardium contains an excess of fluid, portions of which may be clotted. Moderate congestion and interstitial edema are observed in the lungs. The abomasum is frequently distended with large quantities of milk, only a portion of which may be coagulated. The mucous membrane of the abomasum is hyperemic and coated with a thick layer of tenacious mucus.

Histopathology. The most characteristic histopathological changes are observed in the large and small intestines. Sections of the small intestines of calves show extensive pathological changes, with the principal lesions occurring in the mucosa and submucosa. In some sections the necrotic process extends to the muscularis mucosa. Frequently the villi are completely destroyed, presumably by the necrotizing action of the toxin. The lumen of the intestine contains large quantities of erythrocytes, some of which are hemolyzed, fragments of necrotic villi, polymorphonuclear leukocytes, and many bacteria. Gram-positive bacilli, occurring singly or in short chains, are the dominant bacterial organisms. Varying degrees of hemorrhage, vascular congestion, edema, and distention of the lymphatics are noted in the muscularis and subserosa.

The intestinal lesions are less severe in lambs. They vary from catarrhal enteritis and intense hyperemia of the mucosa to hemorrhage and necrosis of the mucosa and lamina propria. Histopathological changes in the abomasum are limited to catarrhal abomasitis, petechiasis of the mucosa, and edema of the submucosa. The mesenteric lymph nodes are edematous and hyperemic, the lymph spaces are filled with fluids and erythrocytes, and the lymph sinuses are distended. Subepicardial and focal myocardial hemorrhages may be noted in the heart. Liver sections are characterized by central congestion and cloudy swelling of the hepatic cords. Frequently the sinusoids are obliterated by the swollen parenchymal tissue. Considerable pigmentation of the cytoplasm may be observed. Microscopically, the kidneys show areas of hyperemia and hemorrhage scattered throughout the parenchymal and stromal tissues. The epithelium of the convoluted tubules is swollen, and the cytoplasm shows considerable granulation.

Bacteriological and toxicological findings. The dominant organism on direct smear of the intestinal content is a gram-positive, nonmotile, medium-sized bacillus, morphologically resembling *Cl. perfringens.* The organism, when incubated anaerobically on blood agar, produces a sharp hemolytic zone around each colony, a stormy fermentation in milk, and conforms to the typical carbohydrate reactions of *Cl. perfringens.*

Bacteriology and toxicology. Bacteria-free filtrates of intestinal contents from naturally occurring cases are lethal to mice when administered intravenously. Toxin present in intestinal filtrates can be neutralized by Types B and C antitoxin. Such results indicate that beta toxin is present.

Strains of *Cl. perfringens* isolated from cases of hemorrhagic enterotoxemia in calves and lambs produce a lethal toxin when grown in modified Brewer's medium as described by Vawter and Records.[13] Filtrates contain a toxin which is neutralized by Types B and C antitoxin.

Diagnosis. Symptoms are not frequently observed because of the rapid course of the disease. The principal findings are a history of sudden death and an acute hemorrhagic enteritis at necropsy. Microscopic examination of a direct smear of the intestinal contents is helpful.

Laboratory diagnosis consists of the following procedure: The intestinal contents are diluted with equal parts of distilled water and filtered through a Seitz or comparable filter. The filtrate is then inoculated intravenously into Swiss mice weighing approximately 20 to 25 gm. The dosage is 0.2 ml. per mouse. If toxin is present, death usually occurs within 30 minutes, but may require 3 hours. Equal parts of toxic filtrate and antitoxin, diluted to contain approximately 200 beta units, are mixed and allowed to stand for one hour at room temperature. Mice are inoculated intravenously with the neutralized mixture, using a dosage of 0.4 ml. per mouse. Control mice are inoculated as before with filtrate only. Death of the control mice and survival of the mice receiving the filtrate antitoxin mixture identifies the intestinal toxin to be the beta fraction of *Cl. perfringens* Type C. If the toxin is not neutralized, further tests are necessary. As an alternative to filtration, the diluted intestinal contents may be centrifuged for one-half hour at 3,000 rpm, and the supernatant used. In such an event it is not advisable to rely upon mouse deaths occurring after three hours.

If toxin is not detected in intestinal contents, cultural methods may be employed in an effort to isolate *Cl. perfringens.* Cultures are typed by the toxin-neutralization test.

Cl. perfringens Type C antitoxin may be used under field conditions as an aid in establishing a diagnosis. The therapeutic and prophylactic effectiveness of antitoxin has been demonstrated by Griner and Johnson[8] and Griner and Baldwin.[14]

Control. Immunological studies conducted by Griner and Baldwin[14] demonstrated that the disease could be controlled by biological products. A whole-culture toxoid was effective prophylactically when administered to the pregnant cow between the third and sixth month of gestation. The active immunity stimulated in the cow was passed via the colostrum to the offspring and provided the offspring with adequate passive immunity during the period of greatest danger. *Cl. perfringens* Type C antitoxin, derived from hyperimmunized horses, was demonstrated to be effective prophylactically when administered to the newborn shortly after birth. It was also effective therapeutically when used in large doses and administered early in the course of the disease.

Ovine Enterotoxemia (*Type D Enterotoxemia*)

Distribution. This disease entity is also known as overeating disease, pulpy kidney disease, braxylike disease, and apoplexylike disease. Type D enterotoxemia is widespread throughout the world's sheep raising industry. Little is known of the disease in goats and, therefore, the following discussion will exclude this species.

All ages of sheep are susceptible to enterotoxemia. It is generally agreed that the most aggressive and vigorous lambs are the most likely victims. The inciting cause is thought to be the ingestion of large quantities of highly nutritive feedstuffs, which results in an indigestion and a static condition of the intestinal tract. Under such conditions it is assumed that, should the animal in question be harboring a toxogenic strain of *Cl. perfringens* Type D, the organisms are provided with a suitable environment and grow rapidly, producing toxin. Under laboratory conditions it is definitely known that toxin can be produced only if the selected media contains nutritives that induce rapid growth. Usually 500 mouse MLD per cc. are found in the contents of the intestinal tract in the region of the jejunum in lambs which have succumbed to enterotoxemia. Baldwin *et al.*[15] demonstrated that lambs weighing approximately 100 pounds were killed by the intravenous administration of 2,000 mouse MLD.

Suckling lambs two months of age or less are frequently victims of enterotoxemia. This is particularly the case in Australia and New Zealand, as well as in the United States. The incidence of the disease has been observed to be greater in single rather than twin lambs, presumably because of the more abundant supply of milk from the ewe. Active immunization of the ewe during pregnancy by means of *Cl. perfringens* Type D toxoid is successful in providing the suckling lamb with passive protection for a period up to two months. Antitoxin is readily transferred via the dam's colostrum and milk to the offspring.

Enterotoxemia losses are common among pastured lambs, particularly during the spring of the year when the pasture is rich in feed value. Any sudden change from poor to rich pasture is apt to precipitate losses. The chronic form of enterotoxemia, characterized principally by a profuse diarrhea and inappetence, is quite commonly observed in outbreaks occurring in pastured animals.

Feeder lambs are subject to losses from enterotoxemia because of the desire of their owners to fatten the lambs as quickly as possible by feeding large quantities of grain concentrates. Losses in nonimmunized animals not infrequently reach alarming proportions (5 to 20 per cent). Unfortunately the animal owner cannot predict which animals are more prone to succumb to this disease. For example, heavy losses may occur when one group of lambs is fed less than a pound of grain per day, whereas the next group may suffer no losses when fed twice that quantity. Prior to the use of biologics to control enterotoxemia losses, the best managed group of feeder lambs was expected to experience a loss of 3 to 5 per cent during a fattening period of three months. It is now common practice to immunize feeder lambs with a whole-culture toxoid, one injection of which provides adquate protection against losses. Approximately 3 million doses of this biologic are used annually. A specific antitoxin is also used widely to stop losses occurring in nonimmunized feeder lambs. Prior to the advent of effective immunizing agents in 1949, the only method of controlling an outbreak of enterotoxemia was to reduce drastically the intake of feed concentrates, thus increasing the length of time and cost necessary to produce a marketable fat animal.

Mature sheep occasionally are affected with enterotoxemia. The extent of such losses is not known in this country. Serological evidence indicates that subclinical enterotoxemia does occur and, logically, if this be the case, the older the animal the greater the likelihood of natural active immunity.

Symptomatology. Acute enterotoxemia is characterized principally by a rapid progressive weakness accompanied by signs of central nervous system disturbance. Listlessness, incoordination, salivation, dyspnea, opisthotonus, convulsions, and coma are the outstanding symptoms. Death generally occurs within four to eight hours after the onset of symptoms. Occasional animals may die in less than one hour. Mortality of affected animals approaches 100 per cent. Specific antitoxin therapy is effective in approximately 50 per cent of the cases if it is initiated early in the course of symptoms. Since death occurs rapidly there is little opportunity for diarrhea to occur in the average case. Animals that live 24 hours or more generally develop a profuse diarrhea.

Chronic enterotoxemia is characterized by a watery diarrhea and inappetence, as well as the sequelae of these symptoms. Nervous symptoms are infrequent. Occasional animals recover and can be demonstrated to contain circulating antitoxin.

Necropsy findings. Animals that die of the acute form of enterotoxemia will generally exhibit the following gross pathology: cardiac hemorrhages most frequently located subendocardially, congestion of the mesenteric blood vessels, petechial and ecchymotic hemorrhages on the thymus, congestion and swelling of the kidneys, congestion of the abomasum and small intestine, and large blotchy-type hemorrhages in the muscles, diaphragm, small intestine, peritoneum, and pleura. The kidneys degenerate into a pulpy mass a few hours after death, which characteristic gives rise to the name "pulpy kidney disease". Not infrequently, only minor pathological changes are observed.

Chronic enterotoxemia is characterized principally by a mild to moderate catarrhal gastroenteritis involving the abomasum and small intestine. Other gross pathology is frequently absent except for secondary complications such as pneumonia.

Control. A specific toxoid and antitoxin are available to control losses from enterotoxemia. Both have stood the trial of time and have been generally accepted by the sheep industry as being effective products.

Discussion

Cl. perfringens Types C and D are the only recognized causes of specific disease entities in this country. The enterotoxemias caused by them are responsible for an undetermined but unquestionably large loss to the livestock industry annually. Particular emphasis has been placed upon the acute form of these diseases, but what of the subacute and chronic enterotoxemia characterized principally by diarrhea? It is possible that loss from these less dramatic forms of enterotoxemia may also be of considerable economic importance. This is only one of many fields of investigation open for exploration.

The study of enterotoxemia as caused by *Cl. perfringens* and possibly other bacteria is in its infancy. There exists little doubt that knowledge gained in the future will reveal that this entity is of greater importance and of wider scope than heretofore recognized.

Summary

The genus *Clostridium* contains only one organism directly associated with diarrheal disease: *Clostridium perfringens* (*welchii*).

Cl. perfringens is classified into six types, each type producing a different toxin pattern. The toxins are antigenically distinct and may be common to more than one type. In the United States Types C and D have been incriminated as the cause of distinct animal diseases associated with diarrhea.

Hemorrhagic enterotoxemia, a disease of suckling calves and lambs, is caused by the toxins of *Cl. perfringens* Type C. In this disease death may occur within

2 to 24 hours after the onset of symptoms. The longer the course of the disease the more likely the development of diarrhea, which is frequently hemorrhagic. Chronic nonfatal cases characterized principally by diarrhea probably occur according to field observations and serological evidence. Beta toxin, the major toxin of *Cl. perfringens* Type C can be readily demonstrated in the jejunem and ileum of animals dead of the disease. Beta toxin is necrotizing and lethal. The necrotizing effect of the toxin is evidenced by extensive severe necrosis of the intestinal mucosa.

Ovine enterotoxemia is a disease of sheep of all ages, caused by the toxins of *Cl. perfringens* Type D. The course of the acute form of the disease is usually four to eight hours and is characterized by severe nervous symptoms, prostration, coma, and death. The chronic type is characterized principally by diarrhea and inappetence. The major toxin of *Cl. perfringens* Type D is termed epsilon and it is necrotizing and lethal. The lesions it produces in the small intestine are less intense than those due to beta toxin.

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GAMMA GLOBULIN DEFICIENCY IN INFANCY*

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Interest in gamma globulin levels in infants was stimulated by the problem of the large number of sudden unexpected deaths in infants that came to the attention of the Westchester Medical Examiner's office, Valhalla, N. Y. These infants, in apparently good health and without any significant history, symptoms, or signs of illness, are usually found dead in a crib or carriage within hours after last being seen or fed. Years ago these deaths were considered the result of strangulation or suffocation, thought to have been brought about by smothering with bed clothes or pillows or by aspiration into the lungs of the contents of the previous feeding. As many of these infants found dead in their cribs have been found lying face up, however, as have been found lying face down. Significant numbers have been found dead during the summer season when no bed clothes are used. Careful post-mortem examinations have usually revealed no evidence of aspiration into the tracheobronchial tree. For these reasons the concept of suffocation has been abandoned. Considerations such as compression of the heart or trachea by an enlarged thymus gland, as well as numerous other theories, have likewise been discarded as untenable.

Analysis of the circumstances surrounding these cases points toward infection, particularly in the respiratory tract, as being the most likely precipitating factor in the deaths of most of these infants. The extensive studies in sudden death in infancy by Werne[1] support this view. In many of these cases, however, the anatomic evidence of infection is either absent or entirely insufficient to account for the rapid course of events, especially in the absence of any previous clinical manifestations.

Eighty-one of these sudden natural deaths in infants under the age of six months were studied by us during the period from 1950 to 1954. These cases were completely autopsied, and post-mortem bacteriological studies were made. No opportunity for virus studies presented itself. The usual post-mortem findings consisted of congestion of the lungs with varying degrees of edema, subpleural petechial hemorrhages, and subepicardial and pericardial petechial hemorrhages. These findings were considered merely terminal and nonspecific events. Post-mortem cultures of nasopharynx, lungs, and spleen revealed a variety of organisms, some of which were pathogens and others not. Careful search for foci of infection or inflammation revealed that of the 81 cases, 26 had significant respiratory infections; 21 had only minimal evidence of focal inflammation, most frequently found in either the upper and/or lower respiratory tracts; and 34 had no significant anatomic findings whatsoever. None of these cases presented any anatomic evidence of intestinal infection. This was to have been expected because cases with a previous history of diarrhea would

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not have been referred to the Westchester Medical Examiner's office as unexplained deaths.

Review of the ages at which death occurred in these 81 cases revealed that the 55 cases with only minimal or negative findings had a peak incidence between 10 and 12 weeks, whereas the 26 cases with significant respiratory inflammation had no such peak incidence (FIGURE 1).

The seasonal incidence indicated that these deaths are more frequent during the time of the year when respiratory infections are most prevalent. In June, July, August, and September there were 12 cases, whereas in November, December, January, and February there were 37 cases.

During the first few months of life an infant is dependent on the antibodies obtained from its mother during fetal life. While the fetal gamma globulin level may exceed the maternal level, the gamma globulin concentration in the infant's serum decreases during the first two months of life and begins to rise after three months, but does not return to a "normal" level until about the age of five years.[2, 3] At about the age of two or three months the infant begins its own synthesis of antibodies. Although there is no direct parallel between antibody titer and serum gamma globulin levels, it is recognized that the gamma component includes many of the antibody globulins. The age between one and three months might therefore be regarded as a critical and transitional period in regard to antibody and immune mechanisms of the infant. Because

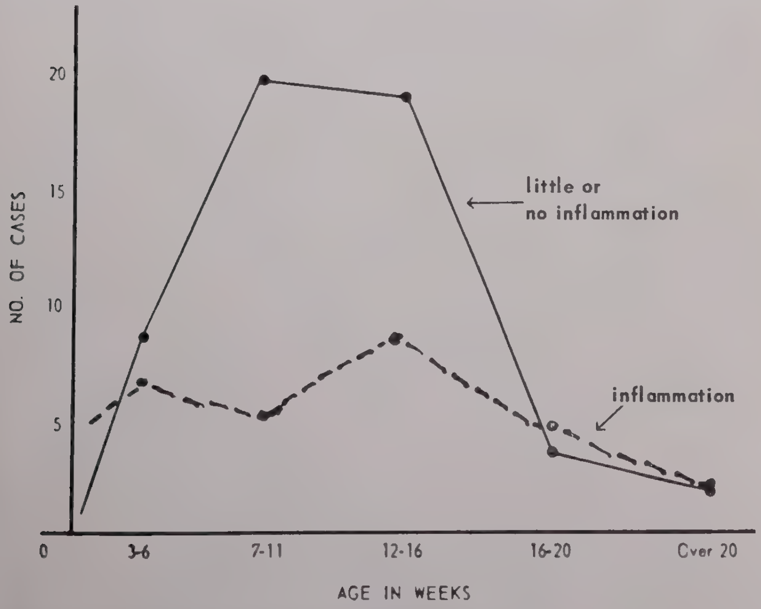

FIGURE 1. The age incidence of sudden unexpected deaths in 55 infants with no significant evidence of inflammation and in 26 infants with significant evidence of inflammation.

the period of the most frequent occurrence of these deaths in infants coincides with this transitional period; because most of the cases occur during the time of the year when respiratory infections are prevalent; and because the majority of these cases revealed either no or, at most, scant evidence of inflammation resembling the response seen in nonimmune animals, it was decided to study post-mortem gamma globulin levels in any new cases that came to our attention.

These studies were begun before we were aware of the condition known as agammaglobulinemia. Bruton[4] was the first to report this entity. In his first case no antibodies could be demonstrated after the administration of typhoid vaccine in the usual manner, and electrophoretic analysis revealed a complete absence of gamma globulin despite the presence of the other proteins in normal amounts. Since his original report Bruton, as well as others,[5, 6, 7] have reported more cases and have elucidated further on this condition. The most recent classification of hypogammaglobulinemia or agammaglobulinemia is as follows:[8] (1) primary agammaglobulinemia, including congenital agammaglobulinemia, adult (acquired) agammaglobulinemia, and transient or physiologic hypogammaglobulinemia; and (2) secondary hypogammaglobulinemia, *i.e.*, that seen in nephrotic syndrome where there is considerable loss of protein.

The infants or children with primary agammaglobulinemia clinically revealed an unusual number of severe bacterial infections, particularly pneumonia, septicemia, and meningitis. It is interesting to note that intestinal infections are not generally included among the complications of this condition. In this situation gamma globulin injections are of prophylactic value, whereas antibiotics are of therapeutic value.

In the present study we were concerned primarily with the transient or physiological form of hypogammaglobulinemia. This differs from the primary form in many respects. The primary form is considered to be a congenital, sex-linked, and permanent defect occurring only in males, whereas the transient form is physiological and, as the name indicates, temporary. Although the incidence of these unexpected deaths in infants is higher in males, there is nothing to indicate any hereditary sex linkage. The primary type at post-mortem examination reveals marked depletion of the lymphoid tissue deposits and complete absence of plasma cells in such sites as lymph nodes, spleen, and intestinal wall. Normal or even excessive amounts of lymphoid tissue are present in the infants with transient hypogammaglobulinemia. In the normal child, antigenic stimulation of the lymphoid tissue results in the appearance of plasma cells containing antibodies specific for the stimulating antigens. Such is not the case in primary agammaglobulinemia.[9] Whether or not antigenic stimulation of a similar nature would result in a significant plasma cell response during periods of physiological hypogammaglobulinemia is not known. In rats or mice proper antigenic stimulation results in a marked increase in the number of plasma cells in the spleen as well as in other sites. Whether or not there are age differences in this response is not known. In the primary type the usual gamma globulin level is below 30 mg. per cent, whereas such extremely low levels are unusual in the transient form.

A fatal case of a generalized vaccinemia has been reported by Keidan *et al.*[10]

TABLE 1

POST-MORTEM SERUM GAMMA GLOBULIN LEVELS (MILLIGRAMS PER CENT) IN INFANTS DYING SUDDENLY AND UNEXPECTEDLY

	Males		Females
Infants with significant evidence of infection	744		731
	814		751
	881		851
Infants without significant evidence of infection	54	279	0
	84	466	96
	101	588	102
	129	804	777
	150	822	820
	180		941

in an eight-week-old baby girl whose electrophoretic analysis showed a deficiency in gamma globulin. This must have been the transient form. The infant had been vaccinated at the age of eight weeks. A small vesicle appeared on the vaccination site one week later. Secondary lesions developed two weeks later, and fresh lesions continued to appear until death, five weeks later. No neutralizing antibodies for vaccine virus could be demonstrated. Other observers[11, 12] have reported the failure to agglutinate antigens in patients with abnormally low gamma globulin levels due to hypoproteinemia.

To date, post-mortem electrophoretic gamma globulin examinations have been performed on 23 cases of unexpected deaths in infants (TABLE 1). In addition, 20 more post-mortem determinations have been performed on infants and children with obvious known causes of death. It may be noted in TABLE 1 that in 9 of the 23 cases the gamma globulin levels were below 200 mg. per cent. In one instance it was completely absent and, in 3 cases, it was below 100 mg. per cent. The normal gamma globulin range in serum is considered to be between 600 and 1200 mg. per cent. The critical levels below which there is a predisposition towards infection, however, is considered to be below 100 mg. per cent. Trouble is usually not encountered in infants during the period of physiological hypogammaglobulinemia. Gitlin` reported a low level of 75 mg. per cent in one case, however, and also noted that in several of the infants the hypogammaglobulinemic state persisted for several months and was associated with recurrent severe infections. Krebs[11] reported a marked depression of gamma globulin levels in hypoproteinemia due to malnutrition. It is conceivable that infants with severe enteric infections with resultant malnutrition and hypoproteinemia could develop marked depletion of gamma globulin, particularly if the enteric infection should occur during the period of transient or physiological hypogammaglobulinemia. It was also interesting to note that in all cases in this study where the post-mortem finding revealed significant inflammatory response, the gamma globulin levels were above 700 mg. per cent. In the other 20 control cases that consisted of infants and young children who died from such obvious causes as trauma, *etc.*, the gamma globulin levels were always above 450 mg. per cent.

From this data it is impossible to conclude that a low gamma globulin level in itself is the significant factor in the pathogenesis of sudden and unexpected death in infancy. One cannot dismiss the fact, however, that the majority of these deaths occur at that period of life during which there is a change-over from the utilization of maternal antibodies to the synthesis of antibodies by the infant. It should also be remembered that antibodies have also been found on other protein fractions such as alpha and beta globulins. Furthermore, cases of recurrent and chronic infections have been reported in infants with hypergammaglobulinemia[13] This indicates perhaps that the level of gamma globulin may not be as important as its make-up or quality. In addition, other mechanisms of natural resistance such as the properdin system, lysozyme, the phagocytic system, and complement must also be considered in studying the problem. Thus it might well be possible to have a condition of low resistance to infections with a normal or high concentration of gamma globulin in the circulation. Studies along these lines in these unexpected deaths in infants are still indicated.

Summary

Study of post-mortem gamma globulin levels in infants who had died suddenly and unexpectedly with no adequate cause revealed that 9 of 17 had levels below 200 mg. per cent. In other infants who have died suddenly and unexpectedly, but have had significant anatomic evidence of infection, the gamma globulin levels have all been over 700 mg. per cent. These results indicate that, although the immune mechanisms may be involved, the level of gamma globulin cannot be considered the determining factor in the mechanism leading to the death of these infants.

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EPIDEMIC DIARRHEAL DISEASE OF VIRAL ORIGIN OF NEWBORN CALVES*

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Recent reviews of the literature on diarrheal disease of newborn calves (Moll,[1] and Lovell[3]) implicate a variety of bacteria in a primary or secondary role. The relative dearth of reports of diarrhea of viral etiology often associated with pneumonia among very young calves (Baker,[4] Jennings, and Glover,[5] Moll and Brandly,[6] and McClurkin[2]) may be attributed to the later development of applicable methods for virus detection and characterization, and to reluctance to deviate from the generally accepted concept that neonatal calf pneumonia-enteritis is a colibacillosis. Recent published reports and other papers in this monograph emphasize and clarify the current progress toward defining the role of viruses in diarrheal diseases of the newborn of various higher animals and man.

An etiological agent of calf pneumonia-enteritis (CPE) prevalent among herds of dairy cattle in Wisconsin has been characterized as a virus by means of serial transmission in calves, using a bacteria-free inoculum, and by serial passage in the gravid uterus of mice and guinea pigs. Further evidence for the viral nature of the agent is provided by its ability to pass through Schleicher and Schuell membrane ultra filters of 50- to 80-mμ effective pore size. Membrane filters of 10- to 50-mμ effective pore size appear to withhold the virus. The inability to grow except in the presence of living cells, resistance to certain antibiotics, and the demonstration of a virus protective substance in colostral whey also attest to the viral nature of the agent. Furthermore, electron microscopy reveals a particulate agent with viral features.

Calf pneumonia-enteritis is manifested by a watery yellow diarrhea and interstitial pneumonia at one to three days of age (McClurkin[2]). Resistance to infection with the virus increases markedly during the second and third days of life, and exposure after 48 hours of age seldom results in the acute fatal disease (TABLE 1).

Exposure of susceptible calves to an aerosol of the virus has proved to be the only uniformly satisfactory method of transmitting the disease. The oral, intranasal, and intravenous routes of exposure have given equivocal results. An infective aerosol has been produced by a No. 166 Vaponefrin nebulizer under 10 lb. of air pressure. A 10 ml. solution of the virus was nebulized into a metal chamber of 14 cu. ft. capacity in which the calf was confined. The exposure time allowed and required for infection was 50 to 60 minutes.

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TABLE 1

INFLUENCE OF AGE ON CLINICAL MANIFESTATIONS OF CPE AMONG COLOSTRUM-DEPRIVED CALVES EXPOSED TO AN AEROSOL OF CPE VIRUS CONTAINED IN LUNG FILTRATE

Calf no.	Age at exposure	Dilution of inocula	Incubation period	Clinical Manifestation	Termination
	in hours		*in hours*[†]		
H54	12	1:20	48	acute	died
H57	3	1:200	24	acute	died
H58	48	1:1000	12	abortive	recovered
H61	18	1:4000	30	acute	died
H62[*]	6	1:40,000	36	abortive	recovered
H63[*]	12	1:40,000	36	abortive	recovered
H64	12	1:16,000	36	acute	died
H66	10	1:32,000	36	acute	died
H67	16	1:32,000	36	acute	died
H76	108	1:10,000		none	remained healthy
H77	96	1:10,000	72	abortive	recovered
H80	96	1:10,000	24	subacute	destroyed
H81	44	1:10,000	24	acute	died
H82	16	1:10,000	24	acute	died
H83	50	1:10,000	72	abortive	recovered
H86	40	1:10,000	24	acute	died
H88	108	1:10,000	24	subacute	recovered
H91	72	1:10,000	36	abortive	recovered
H92	48	1:10,000	12	abortive	recovered
H114	56	1:10,000	48	abortive	recovered
H113	72	1:10,000	24	abortive	recovered
H116	64	1:10,000	36	abortive	recovered
H122	5	1:10,000	24	acute	died

[*] Virus diluted beyond lethal end point.
[†] Calves observed at 12 hour intervals.

Some of the characteristics of survival of the virus were studied by exposing susceptible calves to an aerosol of the virus treated with different chemical agents or subjected to different environmental influences (TABLE 2).

The fact that the virus was stable at 56° C. for 90 minutes and in the presence of diethyl ether for 24 hours permitted preparation of a purified virus suspension from diseased lung tissue. The lung tissue, suspended in saline, was held at 56° C. for one hour, then frozen at −30° C. The fluid was separated from the tissue substance by initiating centrifugation of the tissue in the frozen condition. The resulting supernatant fluid was treated overnight at 4° C. with diethyl ether. The ether fraction was discarded, and a series of low and high speed centrifugation cycles was carried out with the aqueous portion. By alternate suspension in saline and distilled water the virus was separated from the tissue components by differential centrifugation. A Spinco model L centrifuge was utilized for the high speed processing. Low speed centrifugation was carried out at 3,000 rpm in a size 1 type SB International centrifuge. Average centrifugal force was applied 54,450 × gravity for 4 hours, and 59,310 for 3 hours.

The final pellet was resuspended in distilled water. An aerosol prepared from a 1:200 dilution of the purified virus material, with polystyrene latex particles of 132 mμ, was applied to a collodion-treated electronmicroscope grid

TABLE 2

CHARACTERISTICS OF SURVIVAL OF THE CPE VIRUS, USING SUSCEPTIBLE CALVES AS AN INDICATOR

Calf No.	Condition	Clinical signs	Termination
H171	56°C. for 30 minutes	acute	died
H175	56°C. for 60 minutes	acute	died
H181	56°C. for 180 minutes	none	remained healthy
H184	56°C. for 90 minutes	acute	died
H100	56°C. for 360 minutes	none	remained healthy
H122	−20°C. for 6 months	acute	died
H163	−20°C. for 11 months	abortive	recovered
H275	−20°C. for 29 months	none	remained healthy
H274	dried at 25°C. for 12 months	acute	died
H191	phosphate buffer pH 5.4	acute	died
H195	phosphate buffer pH 5.4 stored at −20°C. for 10 days	subacute	recovered
H266	hydrochloric acid—pH 1.3 for 1 hour at 25°C.	acute	died
H267	hydrochloric acid—pH 1.3 for 1 hour at 25°C.	acute	died
H271	NaOH pH 13 for 1 hour at 25°C.	none	remained healthy
H273	formalin 2% for 1 hour at 25°C.	none	remained healthy
H177	diethyl ether at 4°C. for 24 hours	acute	died
H188	diethyl ether at 4°C. for 24 hours	acute	died
H189	diethyl ether at 4°C. for 24 hours	acute	died
H250	chloromycetin 250 mgm., 25°C. for 2 hrs.	acute	died
H270	chloromycetin 250 mgm., 25°C. for 2 hrs.	acute	died
H124	terramycetin 250 mgm., 37°C. for 1 hour	subacute	recovered
H98	penicillin 100,000 units streptomycin—1 gram, 37°C. for 1 hour	acute	died

for one minute. The material was shadowed at an angle of 11° with uranium. The results of electron microscopy revealed a spherical particle of 17 mμ that was not present in normal lung material prepared in an identical manner (FIGURE 1).

When susceptible calves were exposed to an aerosol of the purified virus the ensuing acute fatal disease was the result. These findings together with the filtration results, which suggested an infectious particle of less than 25 mμ, indicate that the particle seen in the slide preparation represents the virus.

Attempts to discover an indicator host other than the calf have been unsuccessful. The virus, however, has been shown to live and propagate in the gravid uterus of the mouse and guinea pig. Signs of the virus infection could not be seen, but when susceptible calves were exposed to the virus contained in the guinea pig or mouse uterine tissues they developed the typical acute disease, indicating the presence of an infection by the virus. The rodent tissues represented the 12th passage in the 2 species, and its lethal titer for calves was 10^{-2}. In contrast, the maximum lethal titer of infective calf lungs obtained was 10^{-4}. With the serum and feces, only undiluted material was lethal.

A substance capable of protecting calves and remaining stable at 56° C. for at least 30 minutes is present in colostral whey but the whey must be fed at least 2 hours prior to exposure in order to demonstrate the presence of the protective substance. Feeding the colostral whey after exposure to the virus

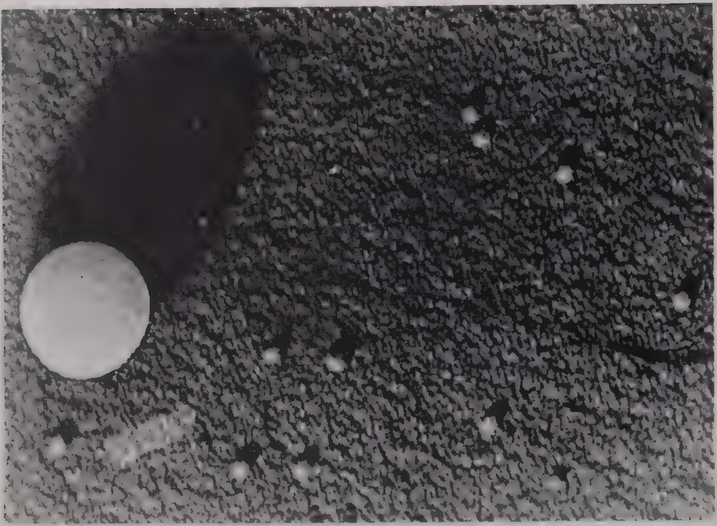

FIGURE 1. CPE virus particle from purified diseased calf lung. The large white ball is polystyrene latex of 132 mμ diameter. ×160,000.

TABLE 3

PROTECTION AFFORDED CALVES BY FEEDING 500 ML. OF COLOSTRAL WHEY BEFORE AND AFTER EXPOSURE TO LETHAL VIRUS

Calf No.	Time administered in relation to exposure to the virus	Clinical signs	Termination
H190	5 hours prior to exposure	none	remained healthy
H219	12 hours prior to exposure	none	remained healthy
H220	8 hours prior to exposure	none	remained healthy
H285	6 hours prior to exposure	none	remained healthy
H214	immediately before	acute	died
H192	1¼ hours postexposure	abortive	recovered
H199	1¼ hours postexposure	acute	died
H202	1¼ hours postexposure	none	remained healthy
H203	1¾ hours postexposure	acute	died
H206	1¼ hours postexposure	subacute	recovered
H216	1¼ hours postexposure	acute	died
H197	4 hours postexposure	acute	died
H198	4 hours postexposure	acute	died

has had very little value (TABLE 3). A virus-inactivating substance in colostral whey has not been demonstrated by the *in vitro* technique. This suggests that the protective power of colostrum may depend upon some factor, in addition to globulins, that augments the host resistance and is manifest only in the presence of the host tissues.

An investigation of the possible presence of the virus in apparently normal bovine animals was undertaken in order to elucidate the probable epizootiology of the disease. Two composite samples of cow lung and two composite samples of calf lung were collected from the local abattoir in September 1953 and in June 1955. These samples were taken from animals purchased for slaughter, and showed no grossly visible lesions. When susceptible calves were exposed to 1:5 and 1:10 dilutions of filtrates prepared from these samples they developed the typical disease, indicating that the virus was present in apparently normal carrier animals. The presence of virus in the lung of apparently healthy animals suggests that they are the reservoir for the virus. Inasmuch as the effective method of experimental transmission is by means of an aerosol, it would appear that the natural method of transmission and spread may be by means of infectious particles passing from the healthy carrier to the susceptible animal, perhaps through the air. The virus could be built up to a lethal concentration in the atmosphere of a barn, and exposure of the newborn calf would take place as soon as it started to breathe. Such factors, rather than low temperatures per se, may account for the increased incidence of calf pneumonia-enteritis during the winter months. Experimental work in which calves were kept at 20° to 30° F. did not indicate that low temperatures lowered the susceptibility of the calves to the virus, nor did the low temperatures enhance the infectivity by means other than aerosol exposure.

In the preliminary work, the problem of securing healthy colostrum-free control calves appeared to be almost insurmountable. As the air-borne nature of the disease and the high frequency of the virus-carrying host became evident, however, emphasis was placed upon securing calves in those seasons of the year when the barns were open and the adult animals were not housed in the barn except at milking time. As an added precaution the calves were separated immediately from their dams and placed outside or in a building not used to house cattle. By this practice a total of 82 colostrum-deprived calves were secured and held as controls for the problem. The controls indicate that under the proper conditions healthy colostrum-deprived calves can be obtained.

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EPIDEMIC DIARRHEAL DISEASE OF VIRAL ORIGIN IN NEWBORN SWINE*

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The knowledge of viral diseases that are primarily diarrheal in young swine is limited at present to one disease. This disease has been called transmissible gastroenteritis of swine (TGE). It is probable that there are other viral agents that cause similar disease in pigs, and it is entirely possible that all of the symptomatology designated as transmissible gastroenteritis is not caused by the same virus. Furthermore, diarrhea may be among the manifestations of other viral diseases, such as hog cholera, that are not considered primarily enteric, and in which serious losses are not limited to newborn swine.

The initial demonstration of the transmissibility of this disease and a partial characterization were reported by Doyle and Hutchings[1] in 1946. Transmissible gastroenteritis has since been studied as part of the North Central Regional Project on Death Losses in Young Pigs† and has also been investigated at the Veterinary Virus Research Institute at Cornell University, Ithaca, N. Y.

Transmissible gastroenteritis has been reported in most or all of the major swine-producing states of this country[2-5] and is recognized as one of the important causes of death losses in newborn swine. It occurs most frequently in sporadic outbreaks that affect the swine on only one farm in a community, although occasionally the disease spreads from farm to farm and becomes a community problem. Outbreaks may occur at any time during the year, but most of the cases are seen in the late winter and spring. Possible contributing factors for this seasonal incidence are that larger numbers of pigs are farrowed during these seasons and that these pigs are usually farrowed in relatively confined quarters such as central farrowing houses or closely placed individual farrowing houses. Under these conditions, losses of several hundred pigs in a week or two are not uncommon. The source of infection on farms in which isolated outbreaks occur has not been determinable in most of our cases. Occasionally it has been found that new swine had recently been introduced into the herd, suggesting that these new animals were carriers of the virus. More often, however, no new swine had been added to the herd for long periods; the last additions usually being boars purchased four to six months previously.

Although transmissible gastroenteritis is often regarded as a disease of baby pigs because of the high mortality rate in this group, all ages of swine may be affected. The manifestations of the disease in swine more than a few weeks of age are quite variable, and infection may be inapparent. In baby pigs, especially those under 10 days of age, the clinical features are quite constant. The incubation period is very short. The first sign, usually vomition, may be observed as soon as 16 hours, usually within 24 hours, and rarely as long as 72 hours after experimental exposure. In naturally exposed herds, where pigs are born in infected quarters, they usually sicken the day after birth. Vomition is followed quickly by a profuse diarrhea which is usually yellowish but may be greenish or whitish in color and sometimes has the appearance of slightly curdled milk. Near the onset a slight elevation of temperature may occur, but most pigs show normal or slightly subnormal temperatures. The pigs lose weight rapidly, become dehydrated, and show an excessive thirst. Affected pigs will consume considerable quantities of water if it is available. The young animals become progressively weaker, and death usually follows two to five days after the onset. In baby pigs that survive, diarrhea usually persists for 5 to 10 days. The mortality rate among newborn pigs in a newly affected herd often approaches 100 per cent.

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† This project, authorized under the Metcalf-Hatch Act of 1952, is being carried out at the Agricultural Experiment Stations of 12 states: Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

Sows with affected litters may show vomition, diarrhea, and a diminution of milk secretion or they may show merely a transient inappetence or often no signs of disease at all. Shoats and fattening hogs on infected premises frequently develop profuse watery diarrhea which spreads rapidly through the drove, affecting most of the animals. In these the course is quite short. Most of the older animals recover in two to five days, with a few in a herd showing diarrhea for as long as a week. Spontaneous recovery of swine over two or three weeks of age is the general rule, and death losses in this age group are rare.

The pathological changes in affected pigs are quite variable and are probably influenced by the presence of secondary pathogens. Affected baby pigs kept in isolation often show no gross changes at death except dehydration, excessive fluid in the intestines, and slight congestion of the mesenteric blood vessels. Other affected pigs may show varying degrees of inflammation in the gastrointestinal tract. There may be moderate to severe hyperemia of the gastric mucosa, especially in the fundus, and the mucosa of any part of the intestinal tract may be congested. Microscopically, there may be necrosis and loss of the surface epithelium and infiltration of the mucosa by neutrophils. The renal medulla is often congested, and the tubular epithelium of the cortex may show albuminous or fatty degenerative changes. No inclusion bodies have been demonstrated.

Transmissible gastroenteritis is serially transmissible in pigs with filtrates that fail to produce growth on a variety of media and culture conditions[1, 6] and with materials treated with penicillin and streptomycin *in vitro*. Bay *et al.*[5] found that the virus was inactivated by concentrations of 0.5 per cent phenol, by 0.05 per cent formalin, and by a temperature of 56° C. for 30 minutes. Suspensions of intestinal tract tissues containing virus kept at room temperature for 10 days or desiccated over sulfuric acid for the same length of time were shown to be inactive. Suspensions treated similarly for 3 days caused the disease in baby pigs. The virus appears to be quite stable when stored in the frozen state. One lot of intestinal tract extract that was shown to be infectious at a 10^{-6} dilution on storage was found to be infective at 10^{-5} after one and one-half years of storage at $-18°$ C.

The virus has not been shown to be pathogenic for any species other than swine, and attempts at propagation in embryonating chicken eggs have been unsuccessful. The virus apparently has an affinity for epithelial tissue, especially that of the intestinal tract. There is a viremia in affected pigs for at least part of the course of the disease, and virus has been demonstrated in most of the viscera. We have found the highest titers of virus suspensions or extracts in the intestinal tract, and high titers have also been found in the kidney.[6] A study recently reported by Young *et al.*[7] gave evidence that the virus multiplies mainly in the intestinal tract. This work also showed that multiplication also occurred in the nasal mucosa of pigs exposed at five days of age and in the lungs of pigs exposed two hours after being taken from the dam by hysterectomy.

The virus is present in the feces during the acute phase and is easily demonstrated by oral inoculation of pigs. It has been recovered for periods from two to eight weeks after inoculation of young pigs by Lee *et al.*[6]

The disease is easily transmitted by contact with affected animals and by oral or intranasal administration of infective material. Keeping healthy pigs in the same room with infected ones but in separate cages has resulted in cross-infection in spite of rigid precautions to prevent transmission other than by air, suggesting that the infection may be airborne.

There are conflicting reports on the results of parenteral inoculation. Bay *et al.*[9] reported transmission of the typical disease by the intracranial, intraperitoneal, intramuscular, and subcutaneous routes. Lee *et al.* reported that pigs inoculated intramuscularly, subcutaneously, or intraperitoneally showed a slight rise in temperature as the only sign of infection, while Young *et al.* found that infection by the intraperitoneal route occurred only following injection of a very large dose of virus.

Field observations have been made in which it appeared that sows exposed three or more weeks before farrowing gave birth to pigs that were resistant to the disease. Bay *et al.*[10] reported immunity in pigs born to sows exposed orally to infective gastrointestinal tract tissue 40 or more days before farrowing. More recent experiments at our laboratory, in which sows were exposed experimentally or naturally, indicate that some degree of resistance may be transmitted from the sow to her pigs. When pigs from these sows were exposed either orally or by contact, most of them developed a diarrhea, but only after a prolonged incubation period. Moreover, the disease in these pigs was relatively mild and most of them survived. This immunity was transmitted through the colostrum, since pigs removed from these sows to isolation quarters before suckling were highly susceptible.

Attempts at transmission of passive immunity by the use of antiserum have largely failed. We have used sera taken from swine at different ages up to one year. These sera were collected from young pigs that had recovered from the disease or from older animals after repeated oral and intravenous exposure to the virus. In various dosages, the sera were given orally, intraperitoneally, or subcutaneously to baby pigs before and at the time of experimental exposure, as well as shortly after birth in a natural outbreak. All of the pigs so treated and exposed, with only one exception, developed the disease. Some lived slightly longer than controls but very few survived. Attempts to confer immunity with gamma globulin from repeatedly exposed swine have met with the same results.

Sera from swine that have recovered from the disease are capable of neutralizing the virus *in vitro*. This neutralizing capacity is the basis for the only serological test for this disease reported thus far.[11] Although this test may be used to identify the virus accurately, its use is limited because of the necessity of using susceptible pigs as test animals. Satisfactory isolation equipment and rearing methods have been developed for the use of baby pigs in virus research,[12] but the fact remains that pigs are a relatively expensive laboratory animal.

At present the field diagnosis of transmissible gastroenteritis is based upon observation of the typical symptoms, especially vomition in baby pigs; rapid spread through a herd and the failure of therapeutic agents usually effective in enteric infections in swine. Further substantiation of the diagnosis may be made by exposing susceptible baby pigs to filtrates or antibiotic-treated suspensions of feces or intestinal tissues of infected pigs.

No therapeutic measures reported have proved to be of any value. It is possible that treatment directed toward replacement of water, electrolytes, and other nutrients would be of value, but in the treatment of swine the economic value of the animal and restraint problems limit the application of this type of therapy.

Control of transmissible gastroenteritis is directed mainly toward prevention of infection. In herds where pregnant sows have not been exposed during the initial outbreak, measures are taken, if facilities permit, to isolate these animals and have them farrow in separate quarters. In some instances it has been found that selling sows due to farrow in the next four to six weeks has been effective by preventing the addition of new susceptible animals during this time. In outbreaks where sows will almost certainly be exposed at farrowing, it may be practical to expose them deliberately with infective materials taken from pigs on the same farm. One report by a practicing veterinarian[13] indicates that control was effected on one farm by this procedure. We deliberately exposed the sows on one farm during an outbreak of transmissible gastroenteritis. Nearly continuous farrowing was practiced on this farm and, because the sows were kept in a pasture remote from the farrowing house, it was likely that they had not been exposed. These sows were fed a suspension of intestinal tracts taken from the affected pigs. No evidence of disease followed this exposure in the sows other than that a slight inappetence for two days was observed by the caretaker. In this case the last death losses due to transmissible gastroenteritis occurred in pigs born 15 days after exposure of the sows.

Summary

Transmissible gastroenteritis is the only primarily diarrheal disease of newborn swine in which viral etiology has been established. It is probably present in enzootic form in all of the major swine-raising areas of the United States. In newborn swine, transmissible gastroenteritis is characterized by a very short incubation period, vomition, diarrhea, rapid dehydration and an extremely high mortality rate. In older swine infection may be inapparent or it may produce watery diarrhea, occasional vomition and, rarely, prostration. Spontaneous recovery within a few days is usual in animals three weeks of age or older.

The observed pathological changes have been mild to severe inflammation of the gastrointestinal tract and congestive and degenerative changes in the kidneys.

The disease is serially transmissible by oral or nasal instillation of bacteria-free filtrates of feces, gastrointestinal tract, lungs, kidney, spleen, or blood serum of infected animals. Of these, the intestinal tract appears usually to have the greatest concentration of virus.

The virus is inactivated by heat at 56° C. for one-half hour, by 0.5 per cent phenol, 0.05 per cent formalin and, after drying at room temperature, for 10 days. It is not affected *in vitro* by penicillin or streptomycin. No species other than swine has been shown to be susceptible to this virus and attempts to propagate the virus in other species have thus far failed.

Field and experimental evidence indicates that some degree of immunity may be transmitted from previously infected sows to their young through colostrum. *In vitro* neutralization of the virus by serum from recovered animals has been demonstrated. Injection of blood serum from repeatedly exposed swine has failed to protect susceptible pigs from the disease.

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PROPAGATION OF TRANSMISSIBLE GASTROENTERITIS VIRUS IN TISSUE CULTURE

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The causative agent of transmissible gastroenteritis (TGE) has been recognized for a number of years as a virus that produces a high mortality in young pigs. Since the original description of the disease by Doyle and Hutchings in 1946,[1] reports have been made from various parts of the United States concerning serious outbreaks of the disease.[2-4] Thus, it has now become common knowledge that the disease occurs wherever swine are raised.

Until recently, however, inoculation of susceptible animals has remained the only means of studying this virus, since no practical serological tests were available. Accordingly, studies were initiated to determine whether the virus could be propagated in tissue culture and, if so, whether a suitable antigen could be prepared for use in serological tests.

Cultivation of Virus

Virus used. An outbreak of transmissible gastroenteritis was observed in 1951, in the vicinity of Ithaca, N. Y. Infective material was secured by sacrificing a sick pig, removing portions of the small intestine, and transmitting the disease to other pigs. This strain of transmissible gastroenteritis virus was designated "New York II."[3] In studies on filterability, a 10 per cent suspension of small intestine from an infected pig was prepared in buffered saline, centrifuged at 3,500 rpm for 10 minutes, and the resulting supernatant was filtered through Seitz EK pad under 5 lb. pressure. A pig was fed 1 ml. of the filtrate. The filtrate produced characteristic signs of TGE. In a further test of infectivity, portions of intestine were removed from this pig and tested in a similar manner in other pigs. All pigs inoculated became ill. The infective filtrate from this tissue was selected as original inoculum for tissue culture passage.

Tissue culture methods. Roller-tube cultures were prepared from pig kidneys in a manner similar to that described for the preparation of monkey-kidney tissue by Dulbecco and Vogt[5] and others.[6,7] Tissues were obtained from pigs ranging from 3 days of age to 16 weeks of age. Initial growth of cells was achieved in a medium consisting of Hank's balanced salt solution 89.5 per cent, horse serum 10 per cent, and lactalbumin hydrolysate 0.5 per cent, pH adjusted to 7.5 with 4.5 per cent $NaHCO_3$. Penicillin and streptomycin were routinely added to make the final concentration of 100 units and 100 μg. per ml. respectively. When a satisfactory growth of epithelial sheet had been established in 5 to 7 days, the initial medium was replaced with maintenance medium consisting of either 98.5 per cent Earle's balanced salt solution, 0.5 per cent lactalbumin hydrolysate, and 1 per cent horse serum[7] or synthetic medium No. 703.[8] The cultures then were ready for virus inoculations and remained suitable for use over a period of 2 weeks without addi-

TABLE 1
EFFECTS OF INOCULATION OF VARIOUS PASSAGE TISSUE-CULTURE MATERIAL ON SUSCEPTIBLE PIGS

Tissue culture passage number	Age of pigs	Route of inoculation	Clinical signs		Serum titer by CCAT	
			Diarrhea	Temp. rise	Pre-inoc.	†Post-inoc.
5	7–9 days	Oral	+	+	0	8
7			+	+	0	4
10			+	–	0	8
15			+	–	0	8
7	16 weeks	Oral	–	+	0	16
15		Oral	–	–	0	8
15		I.M.*	–	+	0	16
26		I.M.	–	–	0	16
27		I.M.	–	+	0	8
28		I.M.	–	–	0	16

* I.M.—intramuscularly.
† Serum collected two weeks after inoculation.

tional fluid change. Filtered virus prepared as mentioned above was inoculated in 0.2 ml. amounts into each tissue culture. After inoculation of virus, tubes were returned to the roller drum for further incubation. Fluid in the inoculated cultures was changed every 4 to 8 days. Serial transfers to fresh tissues were made at intervals ranging from 7 to 14 days by an inoculation of 0.2 ml. amounts of pooled centrifuged supernatant fluids.

Results. By the method described above, virus has been carried for 28 serial transfers with 31 fluid changes over a period of 8 months. No definite cytopathogenic effects on cells were observed in the type of tissue culture system employed. To demonstrate the presence of virus in the system, features of illness produced in young pigs after oral inoculation of tissue culture fluid were used as one criterion. As shown in TABLE 1, each of 4 pigs, 9 days of age, was inoculated orally with 1 ml. of undiluted culture fluid from the 3rd, 5th, 7th, and 15th passage. All developed diarrhea, with a slight rise of temperature in 2 pigs. Another pig given virus from the 5th passage when it was 10 days of age became ill. In another test, 2 pigs, 7 days of age, each were fed 1 ml. of fluid from the 10th culture passage. One pig developed diarrhea which lasted 1 day, and the other showed a slight rise of temperature as the only sign of illness. A pig that received virus in the 9th passage showed no signs of illness. None of the pigs inoculated with tissue culture material died of infection.

In further tests, pigs that were approximately 16 weeks of age were used. In a group of 3 pigs, each was fed 5 ml. of undiluted fluid from the 7th passage. All developed a rise of temperature 2 to 3 days after incubation, but none showed diarrhea. Virus from the 15th passage, when inoculated orally into 2 pigs, did not produce any signs of illness. Virus was obtained from the 15th, 26th, 27th, and 28th passage and 1 ml. of material from each passage was inoculated intramuscularly into each pig. A slight rise of temperature in 2 animals was the only sign of illness observed. These animals again were inoculated 2 weeks after inoculation of tissue culture fluid with TGE virus maintained in pigs. All were found immune. These results, although not conclusive, suggested that TGE virus multiplied in the tissue culture system employed. Attempts then followed to develop conclusive evidence by serological methods.

Conglutinating Complement Absorption Test (CCAT)

In view of the fact that swine sera are "lytic" or "procomplementary" when complement-fixing system with guinea pig complement is used, conglutinating complement absorbing (CCA) system was adopted in which no lysis of cells is involved.

Methods. Antigens were prepared from virus cultivated in roller tubes. After a satisfactory outgrowth of epithelial cells had occurred, the initial medium was removed and the tubes were washed with 1 ml. of synthetic medium No. 703 to eliminate interfering substances such as horse serum and, to each tube, 1 ml. of fresh 703 was added. The cultures then were inoculated with 0.2 ml. of infective fluid from the 2nd or 3rd tissue culture passage. After 6 days of exposure to the virus, the fluid phase was removed, pooled, and centrigued at 2,000 rpm for 10 minutes to remove coarse particles. The resulting supernatant was used as antigen without further treatment. Antigen prepared from the 5th passage virus was found less satisfactory and not used in the test. Control antigen was prepared in a similar manner from noninfected fluid.

Sera were obtained from all pigs before inoculation, either with virus maintained in pigs or with tissue culture virus and, again, two weeks after inoculation.

Technique of the CCAT used for TGE was similar to that described for the study of swine leptospirosis.[9] Twofold dilutions of sera to be tested were mixed with 2 units of lyophilized and freshly reconstituted horse serum complement and undiluted antigen, each contained in 0.1 ml. volumes. The mixture was incubated for one hour in a 25°C. water bath. After incubation an indicator system consisting of 0.5 per cent sheep cell suspension sensitized with 4 units of conglutinin in each 0.1 ml. was added. The final mixture was incubated for 2 hours at 25°C. The results were read after shaking each tube lightly in order to detect the presence or absence of clumping of cells.

Results. As shown in TABLE 2, 9 pigs inoculated orally with original virus developed antibody titers that ranged from $\frac{1}{8}$ to $\frac{1}{64}$ when tested against

TABLE 2
ANTIBODY RESPONSE IN PIGS AFTER INOCULATION WITH TGE VIRUS AS MEASURED BY CCAT

	Immune status	No. of samples	No. of sera showing titers of					
			<1:4	1:4	1:8	1:16	1:32	1:64
TGE infected	Preinoculation	9	9	0	0	0	0	0
	Postinoculation	9			2	2	3	2
Controls		7	7	0	0	0	0	0
		7	7	0	0	0	0	0

tissue culture virus. These sera also were tested against control antigen and found to be negative. None of the 7 control pigs showed any titer during the period of observation. Thus it was shown that virus cultivated in tissue culture could be used as antigen for CCAT. The reliability of the test is suggested by the rise of titers in association with recovery from transmissible gastroenteritis.

The mild nature of infection produced by tissue culture virus and the fact that age resistance developed in test pigs made clinical recognition of infection in individual pigs extremely difficult. The CCAT was concomitantly applied, therefore, to determine whether immunity had been established in the animals following inoculation with tissue culture virus. Representative cases are summarized and included in TABLE 1, where it can be seen that preinoculation sera were uniformly negative, but that post inoculation sera showed antibody titers ranging from 1/4 to 1/16. It is significant that all pigs inoculated intramuscularly developed titers ranging from 1/8 to 1/16.

Discussion

It has been demonstrated that a strain of transmissible gastroenteritis virus was propagated for 28 serial transfers in a tissue culture of pig-kidney epithelium. In this medium, multiplication of virus was not associated with destruction of cells and yet was sufficient to produce antigen in the supernatant fluid that could be used with conglutinating complement absorption methods. This method was applied to detect antibody response in pigs inoculated with transmissible gastroenteritis virus maintained in pigs, as well as response to the inoculation with tissue culture virus. When tissue culture virus was inoculated orally into younger pigs it produced only mild signs of illness in some pigs, but most pigs developed detectable antibodies, thus confirming that virus multiplication had taken place in tissue culture.

There was some evidence of attenuation of virus as a result of tissue culture. None of the pigs died that had been inoculated with tissue culture material when they were 7 to 10 days of age. It is noteworthy that pigs in this age group were highly susceptible to the inoculation of virus maintained in pigs and showed a mortality rate ranging from 60 to 70 per cent.[3] No significant signs of disease were noted in older pigs when inoculated orally or intramuscularly with the tissue culture virus. Thus differences were apparent between the original virus and the virus after it had been propagated in tissue culture.

The report described herein dealt with only 1 strain of transmissible gastroenteritis, and the work on it thus far leaves much to be done. Still to be learned are the optimal conditions for virus multiplication, the relative sensitivity of various tissues other than kidney epithelium, and differences between this and other strains of transmissible gastroenteritis.

Summary

A strain of transmissible gastroenteritis virus isolated in New York State has been maintained for 28 serial transfers in pig-kidney epithelial cells for a period of 8 months. Tissue culture supernatant fluid was used as a source

of antigen for conglutinating complement absorption techniques. With this antigen, the presence of antibodies was detected following infection produced either by original virus or by tissue culture virus.

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EPIDEMIC DIARRHEAL DISEASE OF SUCKLING MICE

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In recent years epidemic diarrheal disease of suckling mice has presented a major problem to many breeders of these animals. Frequently, the losses from this condition have been so high as to cripple production for significant periods of time. Furthermore, those workers employing suckling mice in the course of their investigations have experienced difficulty in carrying out their experiments in the face of an outbreak of diarrheal disease. The condition then is of obvious practical importance to both the breeder and the laboratory worker. In addition, however, the complex etiology involved presents a fascinating challenge to the epidemiologically-minded investigator seeking to develop effective methods of control.

Disease Picture

Epidemic diarrheal disease of suckling mice is a condition affecting these animals during the second and third weeks of life. It is characterized by the sudden onset of diarrhea, which varies from the passage of large amounts of yellowish watery material completely soiling the infected mouse to a mild condition recognizable only by the finding of fecal material adhering to the under surface of the tail. Typically, the disease appears between the 10th and 15th day of life without prodromal signs. Occasionally litters are found in which the young animals present a shrunken and dehydrated appearance as early as the third or fourth day of life. A number of mice die within 2 days of onset; those that survive longer develop darker stools which, as they dry about the anal opening, result in obstipation. Once obstipation has fully developed it is usually fatal unless relieved by artificial means. Perianal necrosis and sloughing are occasionally seen among mice spontaneously recovering from the condition. In mild cases uncomplicated by obstipation, complete recovery within a matter of a week is the general rule. More severe cases, however, that do recover spontaneously may show retardation of growth to the point that stunted litters unsatisfactory for experimental purposes are produced. Thus the outcome for the individual litter may vary from 100 per cent mortality to 100 per cent recovery, with no recognizable sequelae. The adult female shows no recognizable signs of disease at any time.

The disease rarely has its onset before the 7th or 8th day of life. The peak incidence falls between the 10th and 15th day, after which there is a sharp drop. It is most unusual to note the onset of the condition after the mice have reached the 17th or 18th day of life (TABLE 1).[1]

Pathology. Pathological changes appear to be limited to the ileum and the colon. In one epizootic described by Syverton and Olitsky[2] these organs were the sites of a marked inflammatory infiltration with polymorphonuclear cells. Frequently ulcerations were found and, occasionally, even sloughing of the mucosa. Quite a different picture was reported by Pappenheimer and his associates[3, 4] in their study of several severe outbreaks occurring in Boston.

TABLE 1
AGE OF VARIOUS LITTERS AT ONSET OF DISEASE

Age in days	No. of litters	Per cent
8	0	0
9	6	6
10	5	5
11	10	11
12	14	15
13	19	20
14	18	19
15	13	14
16	2	2
17	3	3
18	4	4
19	0	0
20	1	1
21	0	0
Total........................	95	100

Data from Cheever and Mueller.[1]

These authors noted no significant inflammatory changes in either the ileum or colon and, in fact, described no more than a slightly increased cellularity of the stroma of some of the villi. Contrary to the experience of Syverton and Olitsky,[2] however, Pappenheimer and his group[3, 4] described the finding of two types of inclusion bodies that occurred with some regularity in the superficial epithelial cells of the villi. In the first outbreak studied,[3] intranuclear inclusions were observed in 75 per cent of suckling mice suffering from diarrheal disease. They were found only in the epithelial cells of the small intestine and in the mesenteric lymph-adenoid tissue. Both in appearance and in location these intranuclear inclusion bodies resembled those described by Hammon and Enders,[5] by Lawrence, Syverton, Shaw, and Smith,[6] and by Lucas and Riser[7] in infectious panleucopenia of cats. They were not found before the 10th day of life nor after the 15th day. Apparently healthy, control mice of the same age group presented a positive incidence of 5.5 per cent. Since these animals were derived from the same stock and animal colony as the diseased mice, it was impossible to rule out an inapparent infection in the former. Subsequent studies[4] of later outbreaks resulted in the finding of cytoplasmic inclusion bodies in the epithelial cells of the small intestine in a major proportion of mice suffering from either the spontaneous or experimentally produced diarrheal disease. These inclusion bodies were found only during the first few days of clinical illness. They were not found in healthy stock mice of a corresponding age, nor were they produced when susceptible animals were fed intestinal extracts prepared from healthy mice. During the course of the disease the inclusion-bearing cells desquamated in the absence of any significant inflammatory reaction. The two types of inclusion bodies—intranuclear and cytoplasmic—were not found in the same animal. In fact, the intranuclear type of inclusion body was found only in the first outbreak studied; later outbreaks were associated with the finding of cytoplasmic inclusion bodies that had not been observed in the first outbreak. In connection with these findings it should be pointed out that different strains of mice were involved in these later studies.

Etiology. As yet there is no agreement among investigators as to the specific etiology of the disease. In the course of their studies on an outbreak occurring in the breeding colony of the Rockefeller Institute, New York, N. Y., Syverton and Olitsky[2] concluded that a member of the *Salmonella* group was the etiological agent. *Salmonella* organisms have not been isolated, however, with any degree of regularity from other epizootics. Stool examinations for ova and parasites have failed to yield evidence incriminating any recognized pathogenic agent. The finding of two types of inclusion bodies by Pappenheimer *et al.*[3, 4] has suggested that a virus or viruses may be concerned in the etiology of this condition. Additional support to this point of view is lent by the fact that the disease has been transmitted by feeding filtrates of ground-up intestines from the sick mice to susceptible suckling mice presumably free of the disease. The number of these experiments, however, has been relatively small, and the widespread assignment of a viral etiology to this condition is based only on presumptive evidence.

If, as appears likely, the disease is in fact a communicable one, it is quite possible that several unrelated agents may cause the same clinical picture. The markedly different pathological findings reported by various investigators suggest that this may be the case. Furthermore, recent work published on the role of coliform bacilli in the pathogenesis of diarrheal disease of human infants suggests a new avenue of approach. The application of tissue culture techniques to the isolation of enteric viral agents should receive a thorough trial.

Incidence. The disease appears to be widespread among breeding colonies in the United States. The usual story is one of a relatively smoldering infection nearly always present in the colony, although it may not often reach epidemic proportions. Occasionally, however, such a marked increase in incidence may develop that, for all practical purposes, the production of mice for commercial use will be brought to a halt. Some breeders have reported mortality rates as high as 80 per cent. Information from other areas is fragmentary. One investigator,[8] however, has reported that a similar disease occurs in sporadic form in Western Europe, but so far has not reached the high incidence levels observed in this country.

Epidemiology. The disease gives every evidence of being communicable. The appearance of diarrhea in one or two litters of suckling mice in a colony is usually followed by a more or less steady spread of the condition throughout the colonies in an entire institution. Although a slow build-up of cases is the general rule, epidemics of major proportions have developed upon occasion within a few days. Attempts to prevent the spread of the disease from one part of a colony to another by means of various sanitary measures have nearly always met with failure. Once the disease has appeared it has been the common experience to have the entire colony infected within a matter of weeks or months. Little is known about the methods of transmission of the disease. The placing of uninfected suckling mice derived from clean stock in contact

TABLE 2

RESULTS OF TRANSMISSION EXPERIMENTS USING PRESUMABLY INFECTED GUT

Material	No. of litters fed	No. of litters developing disease	Age of mice in positive experiments at time of inoculation	Incubation period
			days	*days*
Filtrate	7	1	8	4
Crude suspension	13	2	9, 3	3, 7
Controls	12	0		

Data from Cheever and Mueller.[1]

with a litter of diseased mice usually results in the appearance of the disease in the newly added animals, provided that they have been exposed during the first week of life.[1] Similarly, the addition of infected mice to newborn litters derived from stock presumably free of the disease has resulted in the development of the disease in the younger mice. As mentioned before, it has been possible to transmit the disease to newborn mice by the feeding of suspensions derived from the intestines of mice showing the typical disease picture. The disease may be reproduced by the feeding of crude unfiltered material. In several instances it has been shown that material rendered free of bacteria by filtration has retained its ability to produce the disease (TABLE 2).[1]

The erratic incidence of the disease suggests that its epidemiology may be relatively complex. That inapparent infections may play an important role in the spread of the disease is a possibility that remains to be investigated. Other hypotheses that have been advanced include the concepts of (1) a dual infection (viral and bacterial agents acting synergistically), and (2) unmasking of latent infections by nonspecific environmental factors. Some of the evidence favoring the latter point of view will be discussed in the following section.

Factors Affecting the Incidence of the Disease

A number of factors, environmental or otherwise, appear to affect the incidence of this disease. The more important of these may be summarized as follows:

Season of the year. It has been the common experience of most breeders that the disease becomes prevalent in recognizable form during the winter months of the year while, quite characteristically, the incidence falls remarkably with the coming of spring. In summer one usually finds relatively little recognizable disease present in the colony (TABLE 3).[9] Some breeders of mice take advantage of this marked seasonal incidence by utilizing the warmer months of the year—April through October—for breeding new stock to serve as the nucleus of the colony for the coming year. With the onset of colder weather and the already-described upswing in the incidence of disease, breeding activities are cut down to a minimum. The explanation for this seasonal variation is not clear. Most animal colonies are maintained under conditions of relatively stable temperatures throughout the year. Relatively crude ex-

TABLE 3

PER CENT OF MICE WEANED, BY MONTH

Period	Born	Weaned	Per cent
Aug.–Sept., 1946	144	125	87.7 ± 2.74
Oct.–Nov., 1946	654	240	37.1 ± 1.89
Dec.–Jan., 1946–47	997	606	60.8 ± 1.55
Feb.–Mar., 1947	868	469	54.0 ± 1.69
Apr.–May, 1947	604	454	75.1 ± 1.76
June–July, 1947	252	222	88.1 ± 2.04
Aug.–Sept., 1947	58	47	81.0 ± 5.15
Total	3,577	2,163	60.5 ± 0.82

Data from Cheever and Mueller.[9]

perimental attempts to affect the incidence of the disease by varying the amount of light received have met with failure.

Humidity. At least one worker[8] has reported that the maintenance of humidity at the 50 per cent level or above results in a significant reduction in the amount of disease, and field trials of this possible method of control now under way have given promising preliminary results. It has been suggested, however, that the beneficial effects of increased humidity are limited almost entirely to the prevention of obstipation rather than to an actual diminution of infection.[10] Control studies will be necessary to answer this point.

Strain susceptibility. There is some evidence that various strains of mice vary in their susceptibility to the disease. In one experiment[9] four strains of mice—Harvard, Tumblebrook (Schwentker), CFW, and C—were kept and bred under identical conditions over the course of the year. Diarrheal disease occurred among the young of all four strains, but was definitely more marked among the CFW mice, as evidenced by the fact that a consistently lower percentage of these mice was weaned in comparison to the others (TABLE 4).[9] Attempts to include a fifth strain—C3H—in the experiment failed because of the extreme susceptibility of the young mice to fatal diarrheal disease. Interestingly enough, an apparently disease-free colony of this strain was established by the delivery of young by Caesarian section at term and the subsequent nursing of these animals by presumably disease-free females belonging to another strain under conditions of rigid isolation. This colony was main-

TABLE 4

PER CENT OF MICE WEANED, BY STRAIN

Strain	Born	Weaned	Per cent
Tumblebrook (Schwentker)	924	546	59.1 ± 1.62
Harvard	821	589	71.7 ± 1.57
C	845	527	62.4 ± 1.67
CFW	987	501	50.8 ± 1.59
Total	3,577	2,163	60.5 ± 0.82

Data from Cheever and Mueller.[9]

TABLE 5
PER CENT OF MICE WEANED, BY LITTER

Litters	Born	Weaned	Per cent
1st	603	221	36.7 ± 1.97
2nd and 3rd	1,599	956	59.8 ± 1.22
4th and 5th	1,375	986	71.7 ± 1.41
Total	3,577	2,163	60.5 ± 0.82

Data from Cheever and Mueller.[9]

tained free of disease for over a year, at which time the experiment was terminated. Other investigators have noted the high susceptibility of mice of strains A and C3H and have reported that mice of another strain—C57BL—were relatively resistant to the disease.[11] Recognizing the possibility that genetic factors may be playing a role in the genesis of this condition, these investigators are carrying out breeding experiments in an attempt to establish a relatively resistant line.

Parity of mother. Several investigators have reported on the effect of parity of mother upon the incidence of the disease. In the case of the 4 strains mentioned above, this effect was definite and consistent throughout the year of observation in that in all 4 strains first litters showed a significantly greater susceptibility to disease than did second and third ones, while fourth and fifth litters escaped even more lightly (TABLE 5).[9] Similar results have been reported by Runner and Palm[12] in working with a colony of C3H mice. These investigators point out the possibility that the age of the female at the time of parturition might be the governing factor. In our experience, however, the age of the female (as distinct from parity) has played no consistently significant role. A second possibility is that the females who have reared or lost at least 1 litter of infected mice have developed a sufficiently solid degree of immunity through inapparent infection to transmit an effective resistance to subsequent litters. Such a resistance might be transmitted through the placenta or the colostrum. Experiments designed to investigate these points yielded evidence supporting the hypothesis that immunity transmitted by the mother is a factor governing the outcome of infection in the litter.[1] These experiments were carried out by the transfer of one half of a newborn first litter to a foster mother who had just borne a second or third litter. Half of the latter litter was transferred in similar fashion to the primiparous female. The adopted mice were accepted well by the foster mothers, and both groups of litters were observed under similar conditions. The results obtained suggested that there was a significant transmission of immunity *in utero* on the part of the multiparous females. Evidence that additional antibodies might be transmitted by the milk or colostrum of these females was less convincing (TABLE 6).[1] A third possibility, that hormonal influences may be playing a significant role, remains to be investigated.

Size of litter. Evidence has been brought forward to suggest that large litters are more prone to the disease than smaller ones.[12] In our mouse colony

TABLE 6

MORTALITY RATES AMONG MICE NURSED BY PRIMIPAROUS MOTHERS AND MULTIPAROUS MOTHERS, RESPECTIVELY

Mice	Nursed by primiparous mothers			Nursed by multiparous mothers			Total		
	Total	No. dying	Per cent	Total	No. dying	Per cent	Total	No. dying	Per cent
First litter	47	35	75	57	34	60	104	69	66
Second and third litters	57	23	40	60	9	15	117	32	27
Total	104	58	56	117	43	37	221	101	46

Data from Cheever and Mueller.[1]

significant differences in the incidence of the disease according to the size of individual litters have not been observed with any degree of consistency. Further experiments on a larger scale are indicated in order to answer this question.

Other factors. A host of other possible factors affecting the incidence of the disease has been suggested by a number of workers. These cover such diverse things as diet, type of bedding, and method of cleaning cages. Consistent results, however, have not been obtained whenever any one of these factors has been tested for its effect on the occurrence of diarrhea, and hence it seems unlikely that such factors are playing a primary role. The possibility that the method of handling breeding animals may affect the mortality rate is under investigation at the present time.[13]

Treatment. As yet there is no convincing evidence that any of the numerous therapeutic measures suggested has exerted a significant effect upon the course of the diarrheal disease.

Control. Efforts made to control the disease in various mouse colonies have on the whole been disappointing. It has been the experience of most workers in the field that, although many factors appear to affect the incidence of diarrhea, no one factor has been found that operated consistently. For a period of time, certain environmental factors may appear to be important in determining the incidence of the disease. Subsequently, however, these same factors may appear to play no role whatsoever. Diarrhea of suckling mice is an excellent example of the well-recognized fact that it is usually impossible to institute effective control measures against a given disease in the absence of knowledge of its etiology. Under these circumstances it is obvious that strenuous efforts should be made to eludicate the etiology and pathogenesis of this condition. It is encouraging, therefore, to be able to report that the newly established laboratory for the study of small animals at the New York University-Bellevue Medical Center, New York, N. Y., has commenced a large scale and exhaustive study of the problem. It is the hope of all concerned that the skilled investigators directing this well-designed and supported study will soon turn up with the answers to our problems. The virus studies initiated under the direction of L. M. Kraft[14] are already yielding encouraging results.

Summary

Diarrheal disease of suckling mice represents a major problem affecting the well-being of mouse colonies in this country today. It is uncertain as to whether it represents a single disease entity or, perhaps more likely, a series of separate or overlapping syndromes. The etiology of the condition remains undetermined. The condition is almost surely infectious, and both pathological observations and transmission experiments suggest that a virus or viruses may be playing a significant role in the pathogenesis of the disease. The possibility remains that secondary bacterial invaders may have a significant effect upon the outcome of the infection. Other factors exerting a recognizable effect upon the incidence of the disease are: (1) strain of mouse, (2) parity of female, and (3) season of the year. Other possible but less-established factors are degree of humidity and size of litter. In the absence of definitive knowledge concerning the etiology and pathogenesis of this condition, attempts to control the disease are empirical and so far have been relatively unsuccessful.

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DIARRHEA IN PUPPIES CAUSED BY DISTEMPER VIRUS

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Diarrhea is a frequent occurrence in puppies. At times it is related to an abrupt change in diet. Sometimes it is associated with parasites, certain bacteria, or other microbial forms. Often there is no definitive etiological basis. Distemper is the only viral infection studied thus far in puppies in which diarrhea occurs.

Dogs affected with the virus of canine infectious hepatitis may have bloody stools, but this apparently is the result of multiple hemorrhages caused by viral action on endothelial cells. Enteritis does not occur in infectious canine hepatitis, and the deviation from normal in the character of the feces is caused by systemic infection.

In distemper, however, there is an enteritis present. Distemper virus has been demonstrated in feces, and the presence of cytoplasmic inclusion bodies in epithelial cells lining the stomach and intestinal tract lends support to the idea that distemper virus by itself produces enteric pathology and diarrhea, although other signs of illness occur, indicating systemic, and occasionally neurological manifestations. Whether caused by action of the specific virus alone or as a complication, diarrhea is seen so frequently that it must be considered a part of the clinical syndrome of distemper.

In their initial studies, Dunkin and Laidlaw[1] thought it possible that bacteria were complicating distemper virus infection and causing the diarrhea. In the absence of specific studies, however, their suggestion must, of course, remain only a possibility.

In their studies on distemper Dunkin and Laidlaw reported that the majority of 70 dogs showed diarrhea throughout the course of illness and that in the second period of the diphasic febrile reaction the diarrhea frequently was profuse, with slimy feces containing small streaks of blood and having an offensive odor. Diarrhea continued after fever had subsided, and the animals appeared to be recovering. The mortality rate in their dogs was 10 per cent, and the majority of these showed nervous manifestations before death. The ages of the dogs used by Dunkin and Laidlaw were not reported.

In our early studies on distemper, 142 dogs were inoculated with virulent virus derived from three different sources. Of these 122 dogs were given material designated "Snyder Hill strain," while the remaining 20 dogs were given one of the other strains of virus used. Cross immunity studies showed all strains to be antigenically similar. Until our disease-free colony of dogs could be established, the dogs used in our earlier studies on distemper were obtained from large litters raised on farms in the vicinity of Ithaca, N. Y. These dogs varied in age from 12 to 20 weeks. Most of the dogs showed diarrhea as did those observed by Dunkin and Laidlaw.

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Certain variations in response were noted among the individual dogs infected by the same route and with the same amount of virus. In an effort to define the specific factors responsible for this variation, later studies were planned in which not only the amounts of virus were controlled, but the entire life history of each puppy was known. These later studies are still in progress and, although incomplete as yet, the findings seem to have a bearing on the aims of this monograph, that is, a better understanding of diarrhea in infants.

Pathogenic Effects of the Snyder Hill Strain of Distemper Virus in Farm-Reared Dogs

Since the Snyder Hill strain of distemper virus[2] was used to inoculate puppies of defined origin, it seemed worthwhile to record what effects had been observed in other types of dogs in order to have a basis for understanding certain signs of infection. The dogs used were obtained from farms. Their ages varied from 12 to 20 weeks, and the status of immunity to distemper of their mothers was unknown. Inoculations of virus were made either intracerebrally, intravenously, subcutaneously, intranasally, or by exposing the dogs to contact with others that had been infected. In all dogs, inoculum consisted of a 10 per cent suspension of brain or of spleen given intracerebrally in 0.3 ml. amounts to each dog and, for other routes, in 1 ml. amounts to each dog.

As shown in TABLE 1, of 60 dogs inoculated intracerebrally, 51 showed epileptiform convulsions, 1 developed myoclonus, 5 died without observed neurological signs, and 3 showed generalized signs of illness only. Of 32 dogs inoculated intravenously, 8 showed epileptiform convulsions, while 6 developed myoclonus and recovered. Of 11 dogs exposed by contact, 4 showed epileptiform convulsions and 2 myoclonus. Of 4 inoculated intranasally, none showed neurological signs.

Clinical features. Dogs given virus intravenously usually developed an elevated temperature 2 days after inoculation, although in some the onset was delayed as long as 8 days. Similar incubation periods were found for dogs inoculated intracerebrally, although an occasional dog failed to show a temperature elevation until the onset of nervous manifestations. Elevated temperatures in dogs inoculated subcutaneously developed in about 5 days, and after 6 days in those inoculated intranasally or exposed by contact. Usu-

TABLE 1

NEUROLOGICAL MANIFESTATIONS SHOWN BY DOGS INOCULATED WITH THE SNYDER HILL SERUM OF DISTEMPER VIRUS

Route of inoculation	Epileptiform convulsions	Myoclonus	Death without observed neurological signs	Total
Intracerebral	51/60	1/60	5/60	57/60
Intravenous	8/32	6/32	0/32	14/32
Subcutaneous	3/12	2/12	0/12	5/12
Intranasal	0/4	0/4	0/4	0/4
Contact	4/11	0/11	0/11	4/11

Dogs were observed for a period of time longer than 45 days. Numerator indicates number of dogs affected. Denominator indicates number of dogs inoculated.

ally the temperatures followed a diphasic pattern, although some remained high throughout the course of illnes. Leukopenia was found in most dogs, and the leukocyte count remained low throughout the acute phase of illness. The usual catarrhal signs such as congestion of the conjunctival membrane and a serous discharge from the eyes and the nose developed shortly after the onset of fever. Later, diarrhea developed in most of the dogs. A few animals showed signs of pneumonia. Except for an occasional dog that showed myoclonus and recovered, all animals that developed marked neurological complications died. All others recovered except a few that developed pneumonia.

Most of the dogs with nervous manifestations had a syndrome characterized by several or all of the following: depression, myalgia, myoclonus, incoordination, circling, epileptiform convulsions, coma, and death. The epileptiform convulsions usually occurred 1 to 2 days before death.

Pathology. Brains from 31 dogs that showed neurological manifestations furnished material for this study. For comparative purposes, there were included brains from 2 uninoculated dogs and from 3 inoculated intracerebrally with virus inactivated by heating a 20 per cent suspension of brain from an infected dog at 60° C. for 3 hours. After autopsy, brains were removed and 22 of them were placed in a large volume of 10 per cent aqueous formalin solution for about 1 week at room temperature. After fixation, thin slices of tissue were cut from various parts of the brains. In the preparation of brains from 3 dogs that had had convulsions and from the 2 uninoculated dogs, thin slices were cut, placed in Susa fixative and in 10 per cent formalin, and kept in the refrigerator for 24 hours. The brains from the other dogs that had shown nervous manifestations and from the 3 that had been given inactivated virus were placed in a large volume of 10 per cent aqueous formalin immediately after euthanasia and refrigerated for 1 week; then thin slices were cut. After fixation, slides were prepared, stained with hematoxylin and eosin and, in some cases, with azure eosinate and Mallory's phosphotungstic acid hematoxylin. Also frozen sections of formalin-fixed cerebellum from some infected dogs and from 5 control dogs, were chromated for 3 days and stained by Lillie's technique for myelin and degenerated myelin.

The cerebellums of all the 31 dogs that developed nervous manifestations showed perivascular cuffing, nonsuppurative leptomeningitis with variable accumulations of mononuclear cells, neuronal degeneration, and vacuoles in the white matter. Many Purkinje cells showed degenerative changes. Numerous cells showed pyknosis, while other cells appeared swollen and their Nissl granules were small and indistinct. Some Purkinje cells had a zone free of Nissl granules around the nucleus, and the nuclei of some cells had migrated to the edge of the cell. Other Purkinje cells had faded so that they were almost unrecognizable and apparently were undergoing cytolysis. Neuronophagia was not recognized. Vacuoles were seen in the white matter of the cerebellum of inoculated dogs. Some similar small vacuoles, however, were found in the control dogs. The vacuoles did not have a perivascular distribution. Degenerated myelin was not demonstrated in frozen sections of cerebellum from dogs that were sacrificed 7 to 16 days after inoculation. Gliosis was seen in the cerebellums of a few dogs and, in general, was most marked in dogs that developed nervous manifestations 22 or more days after inoculation or contact with infected dogs. Uninoculated dogs and dogs inoculated with heat-inactivated Snyder Hill virus showed none of these changes.

Numerous gitter cells and intranuclear inclusion bodies in glial cells were found in the cerebellums of the 2 dogs that developed neurological disturbance after the longer intervals, 22 and 29 days. Tissues suitable for frozen sections and myelin stains were not available from these dogs, but the gitter cells indicated a demyelinating process. Inclusion bodies were not found in the cerebellums of the dogs that developed encephalitis in the shorter intervals after inoculation, although it is possible that exhaustive search might have shown some present.

Pathological Effects of Distemper Virus on Puppies of Known Age from Immunized and from Distemper-Free Mothers

Method. Over 2 years ago a colony of beagles was started at our institute. The parent stock was derived by Caesarian section, deprived of colostrum, and reared on Esbilac* until they were old enough to eat commercially prepared dog foods. Repeated tests in these dogs failed to reveal any serum-neutralizing antibodies for distemper virus and, therefore, the dogs were considered disease-free.

Having thus obtained a suitable breeding colony, work was begun to determine the effects of a given amount of the Snyder Hill strain of distemper virus when inoculated intravenously into puppies of different ages. In the study, a litter of puppies was weaned at six weeks of age, divided into two groups, and each group was placed in a separate isolation unit. Accumulated totals on 8 litters thus far showed that 18 puppies were inoculated when 6 to 10 weeks of age, while a total of 10 were inoculated at 12 to 16 weeks of age. The study was repeated in immunized dogs, mostly with beagles of our own breeding, immunized by exposure to dogs infected with distemper, although a few puppies were from farm dogs inoculated at 5 to 10 weeks of age and 22 at 12 to 16 weeks of age. In these groups, 6 puppies in the 12- to 16-week-old group were litter mates to the 14 dogs in the 5- to 10-week-old group.

Each puppy was inoculated intravenously with 1 ml. of a 10 per cent suspension of spleen from a dog that had been infected with the Snyder Hill strain of distemper virus. After inoculation, daily temperatures were taken and every animal was observed for signs of illness. Each dog was weighed weekly. Animals that died were autopsied, and tissues were saved for histological examination. All dogs surviving for a month were inoculated with the Snyder Hill strain again, either intracerebrally or intravenously, in a test for immunity. In addition, blood was collected and serum obtained from each dog before inoculation and again two or more weeks later unless the animal died in the early stages of infection. Each serum was tested for neutralizing capacity against the Onderstepoort strain[3] of egg-adapted virus in eggs. In this test[4] a virus suspension containing 100 to 500 infectious doses of virus was mixed with each twofold dilution of serum, placed in a refrigerator for 2 hours, and then inoculated into eggs that had been embryonated for 7 days. For every dilution of serum tested, a group of 5 eggs was inoculated with 0.2 ml. on the chorioal-

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* Manufactured by The Borden Co., New York, N. Y.

TABLE 2

RESPONSE OF PUPPIES OF VARIOUS AGES AND FROM IMMUNE AND SUSCEPTIBLE DAMS TO SNYDER HILL STRAIN OF CANINE DISTEMPER VIRUS*

Status of dam	Number of puppies	Age in weeks	Some effects observed			
			Elevated temp.	Diarrhea	Weight gain	Survival
Non-immune	18	6–10	18	18	0	3
	10	12–16	10	4	7	10
Immune	14	5–10	4	2	12	12
	22	12–16	21	11	17	17

* Inoculated intravenously with 1 ml. of 10 per cent suspension of virus-laden spleen.

lantoic membrane. After a further incubation of 6 days the eggs were examined for lesions. Failure to find lesions indicated neutralization.

Results. In TABLE 2 it can be seen that all dogs in both groups from distemper-free mothers developed elevated temperatures. All puppies in the younger age group showed diarrhea, and most of them had blood-tinged feces. In contrast, only 4 of the 10 in the older group showed diarrhea. All of the younger dogs lost weight, but this occurred in only 3 of the older age group. Of the 18 pups in the younger age group, 15 died 9 to 20 days after injection, while all the older puppies survived. On autopsy, the most constant pathological lesion was a hemorrhagic enteritis. Preinoculation sera of all dogs failed to neutralize virus in a dilution of 1:2.5. The 3 survivors in the younger age group and all the dogs in the older group developed serum-neutralizing antibodies.

Of 14 dogs in the 5-to-10-week age group from immunized dams, 4 had elevated temperatures, and 2 developed diarrhea and died 11 days after inoculation. When autopsied, both of these latter 2 dogs showed hemorrhagic enteritis. All the other dogs survived and grew normally. The preinoculation sera of the 4 puppies that showed signs of illness failed to neutralize virus at a dilution of 1:2.5. The other 10 puppies had demonstrable serum antibodies at the time of inoculation. In the older group of 22 dogs, 21 developed a diphasic temperature reaction and, of these, 11 had diarrhea. Seventeen of the older dogs survived, and these gained in weight. Five dogs in the older group lost weight and died following epileptiform convulsions. The preinoculation serum of each dog failed to neutralize virus, whereas the convalescent sera of all survivors had demonstrable serum-neutralizing antibodies. On histological examination of various tissues, the most striking lesion was seen in the brain, and was a demyelinating encephalitis characteristic of distemper.

Summary

It would appear that diarrhea and death are important features of distemper infection in puppies, whose blood serum contained no neutralizing antibodies when they were 6 to 10 weeks of age and came from distemper-free mothers. A less severe infection occurred in their litter mates when, at 12 to 16 weeks of age, they were given the same virus and kept under similar conditions. The majority of puppies less than 12 weeks of age and from mothers immune to distemper showed no signs of illness when given virus, but those over 12 weeks of age developed signs of illness of a degree of severity similar to puppies from the nonimmunized mothers.

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MIYAGAWANELLA BOVIS INFECTION IN CALVES

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Diarrhea in the newborn calf is a serious problem of the dairy industry and, in spite of much study by many workers, the etiological complexities of the condition have not yet been unravelled.

The purpose of this paper is to discuss the disease-producing potential of an agent that is found naturally in the intestinal tract of calves and that belongs to the psittacosis-lymphogranuloma group of viruses. The agent was first described by York and Baker (1951) and provisionally designated by them *Miyagawanella bovis.*

Studies on Miyagawanella bovis

Isolation. In the course of studies with various rickettsial agents, it was found that when fecal material from apparently normal calves was inoculated into guinea pigs intraperitoneally, a febrile response was produced. Serial transfers of suspensions of infected guinea pig liver and spleen to other guinea pigs produced elevated temperatures. It appeared that an infectious agent had been isolated. Since tissues from these guinea pigs were bacteriologically sterile, a virus was suspected and subsequently identified as a new member of the psittacosis-lymphogranuloma group. Subsequent to the original recovery of the virus, strains have been isolated in a similar manner from the feces of calves in Indiana, California, and Montana.

Characteristics of M. bovis. In addition to causing a febrile reaction in guinea pigs, but not death, initial studies showed *M. bovis* produced pneumonia in mice, although it was not until after 20 successive transfers that the virulence was sufficiently enhanced to cause death of inoculated mice. The inoculation of the virus into young cats, dogs, and swine caused no significant illness or pathological changes. When injected into the yolk sacs of embryonated hens' eggs, however, it usually killed the embryos in 4 to 6 days. It also grew in the allantoic cavity and on the chorioallantoic membrane. Films prepared from the peritoneal exudate of infected guinea pigs and stained by Macchiavello's method occasionally showed small coccoid red-staining bodies in a few of the mononuclear cells that constituted the cellular portion of the exudate. Films from infected yolk sacs, when stained by the same method, revealed numerous small red-staining coccoid bodies that were located both intracellularly and extracellularly, singly or in clumps. Numerous bodies of similar appearance also were seen in impression films of lungs from infected mice. Individual bodies markedly resembled the elementary bodies of the psittacoid group of organisms, but only occasionally were they seen in plaques or vesicles described as part of the developmental cycle of this group.

Further study showed elementary bodies with an average diameter of 350 mμ were found in electron micrographs. These particles had the morphology of members of the psittacosis-lymphogranuloma group of viruses as reported

TABLE 1

COMPARISON OF SEVERAL STRAINS OF *MIYAGAWANELLA BOVIS* WITH EACH OTHER AND WITH OTHER AGENTS BY THE COMPLEMENT-FIXATION TEST

Agents tested	Antisera						
	Strain 1	Strain 2	Strain 3	L.G.V.	F.P.	*c. bur.*	Normal g.p.
Strain I	1:128	1:16	1:64	1:64	1:8	Neg.	Neg.
Strain II	1:32	1:32	1:64	N.T.	N.T.	N.T.	Neg.
Strain III	1:64	1:16	1:32	1:64	1:8	Neg.	Neg.
L.G.V.	1:32	N.T.	1:8	1:64	1:4	Neg.	Neg.
F.P.	1:32	N.T.	1:8	1:32	1:8	Neg.	Neg.
C. burnetii	Neg.	N.T.	Neg.	N.T.	Neg.	1:32	Neg.
R. prowazekii	Neg.	N.T.	Neg.	N.T.	Neg.	N.T.	Neg.
R. rickettsii	Neg.	N.T.	Neg.	N.T.	Neg.	N.T.	Neg.

Symbols: L.G.V.—lymphogranuloma venereum; F.P.—feline pneumonitis; *C. bur.*—*C. burnetii.*

by Rake, Hamre, and Groupe, (1946). The virus regularly passed through a Berkefeld V filter, but only in 1 attempt out of 3 did it pass through a Berkefeld N filter. Centrifugation studies indicated that the infective particle sedimented at the same rate as the elementary bodies. The agent was found to survive a storage period of 3 days at 37°C., 10 days at room temperature, and 1 month at 4°C., while storage for 1 year at 70°C. resulted in no loss of activity. Tests in embryonated hens' eggs indicated that penicillin and Aureomycin had a pronounced inhibitory effect on the virus, while sulfonamides had little or no effect.

Complement-fixing antigens were prepared for several strains of this calf agent and compared by cross-serological tests with antisera prepared against each of these strains. Comparisons also were made with antigens and antisera of feline pneumonitis and lymphogranuloma venereum, both recognized viruses of this group of organisms. Antisera or antigens of *Coxiella burnetii* and 2 Rickettsia were included. The results are summarized in TABLE 1.

As can be seen from this table, the various strains of the calf agent appeared identical by reciprocal cross-serological tests. Positive cross-serological reactions were also demonstrated with feline pneumonitis and lymphogranuloma, but no cross reactions occurred with any of the Rickettsia used. The growth of this agent in eggs, its staining characteristics, and the sharing of common complement-fixing antigens indicated that it belonged to the psittacosis-lymphogranuloma group of viruses. Other studies, such as neutralization tests with hyperimmune sera and the type of response obtained in various experimental animals suggested that the agent was a new member of this group.

The Disease in Calves

Because of the widespread distribution of *M. bovis*, the majority of animals used in these investigations came from a herd reared and maintained in strict isolation at the Virus Research Institute at Cornell University. This herd was found to be free from infection with the agent under study, both by serological

TABLE 2

HISTOPATHOLOGY AND VIRUS ISOLATION IN FIVE CALVES INFECTED WITH *MIYAGAWANELLA BOVIS*

Calf No.*	Isolation attempts								Histopathology
	Duodenum	Jejunum	Ileum	Cecum	Colon	Spleen	Liver	Feces	
2-32	−	−	−	+	−	−	−	+	None
2-33	−	−	−	+	−	−	N.T.	+	None
2-45	+	−	+	−	−	−	−	+	None
2-48	−	−	+	+	+	−	−	+	None
2-49	−	+	−	+	−	−	−	+	None

N.T.—not tested.

* Calves 2-32 and 2-33 were naturally infected while 2-45, 2-48, 2-49 were infected experimentally.

tests and by repeated inoculation of fecal material into guinea pigs. In some tests, newborn calves were obtained from a dairy farm where the virus had been isolated from older calves and where serum samples from adult animals contained demonstrable antibodies.

During these studies, all the experimental calves were held in strict isolation in separate tile-lined units to prevent cross-contamination from one animal to another.

Calves 4 to 9 months of age. Calves from the Cornell herd were infected by feeding a 10 per cent suspension of infected yolk sac. No signs of illness were observed except a transient leukocytosis above 30,000 lasting from 1 to 4 days. Three animals and 2 naturally infected calves were killed, and their tissues and portions of the intestinal tract examined for lesions. Suspensions of these various tissues were used for virus isolation by inoculations into guinea pigs. The results of these studies are summarized in TABLE 2.

As can be noted in this table, the agent was isolated from one or more areas of the intestinal tract from each calf, although no isolations could be made from the other organs tested. Since it was probably impossible to wash the intestinal contents completely from the crypts of the mucosal wall, this study indicates primarily that infection can occur high in the intestinal tract, but it also suggests that the virus may be produced in all portions of the tract. The virus could be recovered from fecal material of all the inoculated calves and, in the case of those that were not killed, it persisted in the feces for at least six months after inoculation.

In field studies, fecal samples and sera were collected from a number of animals on a group of farms around Ithaca, N. Y. Approximately 60 per cent of the animals were found to be excreting the virus. These naturally affected animals continued to excrete the virus for at least six months after the initiation of these studies. Subsequently, viruses of the same type have been isolated from the fecal material of calves in Indiana, California, and Montana.

Newborn calves. Work with older calves suggested that although the virus grew in the intestinal tract, it only produced an inapparent infection. Nevertheless, its effect in newborn calves was tested. For this work, calves from both the disease-free herd and from an infected herd were used.

The animals from the infected farm were removed at birth to an isolation unit and treated in the following manner:

(1) Five calves obtained from this source received no colostrum but were inoculated with 150 ml. of serum obtained from the disease-free herd. At about 24 hours of age, the calves were each fed 10 ml. of a yolk-sac suspension of *M. bovis.*

(2) Five calves were fed colostrum from their dams for 1 day, and then fed 10 ml. of the yolk-sac suspension.

(3) Two calves received no colostrum but were also given 150 ml. of serum. They were not fed virus, and were used as normal control animals.

The calves from the disease-free herd were handled in much the same way, except that all the calves were allowed to receive colostrum of their dams for the first 24 hours of life. After that the various treatments were:

(1) Five calves were fed 10 ml. of a suspension of virus.

(2) Five calves, in addition to receiving colostrum of their dams, were also fed colostrum obtained from a cow having circulating antibodies against this agent. These calves then were fed virus suspensions.

(3) Five calves were held as normal uninoculated control animals. The results of the inoculations of these groups of calves are summarized in TABLE 3.

As can be seen from this table, of the 5 calves obtained from the infected farm but receiving no colostrum, all had a febrile response, a marked leukocytosis, and diarrhea. Three out of the 5 died on the 5th, 8th, and 17th days respectively, following inoculation. The 5 animals that received colostrum from immune mothers had only a slight febrile response and leukocytosis, and no diarrhea. The 2 calves that were given neither colostrum nor virus remained normal throughout the period of observation.

The 5 calves from the disease-free herd that received the colostrum of their

TABLE 3
EXPERIMENTAL INOCULATION OF CALVES WITH YOLK-SAC SUSPENSIONS OF *MIYAGAWANELLA BOVIS*

Source of calf	History of calf	Inoculation	No. inoc.	Illness observed	Death	Virus recovery
Infected dairy	No colostrum serum I.V.*	Fed 10 ml. y.s. susp.	5	Fever, leukocytosis, diarrhea	3/5†	Feces of all: kidneys of 2
Infected dairy	Colostrum of dam	Fed 10 ml. y.s. susp.	5	Slight fever, leukocytosis	0/5	Feces of all
Infected dairy	No colostrum serum I.V.	None	2	None	0/2	None
Disease-free herd	Colostrum of dam	Fed 10 ml. y.s. susp.	5	Fever, leukocytosis, diarrhea	0/5	Feces of all
Disease-free herd	Colostrum of dam and colostrum of infected cows	Fed 10 ml. y.s. susp.	5	Slight fever & leukocytosis	0/5	Feces of all
Disease-free herd	Colostrum of dam	None	5	None	0/5	None

* Serum obtained from disease-free herd and contains no antibodies against *M. bovis*; inoculated intravenously.
† Numerator—No. dying; denominator—No. inoculated.

dams also had a febrile response, leukocytosis, and diarrhea, but the response was not as severe as in the calves that had no colostrum (above), and none died. The 5 animals obtaining colostrum of their disease-free mothers as well as colostrum from an immune cow showed no response to the feeding of this virus. Similarly, the 5 calves that received colostrum but were not inoculated remained healthy throughout the entire course of experimentation.

From all the animals inoculated, virus could be recovered from feces, or from the intestinal tracts of those that died. The virus was also recovered from the kidneys of the 2 calves that died. Histopathological examination of these kidneys showed a focal interstitial nephritis, whereas kidneys free from the virus showed no lesions. The calves that showed a clinical response following inoculation had no complement-fixing antibodies in this serum before inoculation, but all those surviving developed antibodies. All of the calves receiving colostrum from immune mothers and not responding clinically to the feeding of the virus had low but demonstrable complement-fixing titers at the time of inoculation.

Comment. The work reported in this paper, although limited in the number of animals used, indicates the disease-producing potentialities of *M. bovis*. Further studies must be undertaken, however, to correlate the results of this experimental work with the occurrence of diarrheas of the newborn calf as it develops naturally on the farm. Until this is done, it is not possible to determine whether this virus is one of the agents responsible for calf diarrhea.

Summary

A virus has been isolated from the feces of over 60 per cent of calves from various dairy farms in New York and has been recovered from calves in Indiana, California, and Montana. This agent has the characteristics of a member of the psittacosis-lymphogranuloma group of viruses and has been provisionally designated *Miyagawanella bovis*. Experimental feeding of this agent in calves 4 to 9 months of age produced no illness, but a shedder condition could be readily established that lasted at least 6 months.

With newborn calves, diarrhea and, at times, death occurred from feeding the virus, providing colostrum was withheld, or if colostrum was obtained from nonimmune mothers. Colostrum from immune mothers prevented illness in the calf. Regardless of whether or not colostrum was fed, all inoculated calves that survived became carriers of the virus.

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DIARRHEA OF NEWBORN AND OLDER INFANTS DUE TO VIRUSES

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Rice *et al.*,[1] in 1937, suggested that "epidemic diarrhea of the newborn" is a specific disease entity. This opinion was based on (1) the similarity of the clinical course in the patients involved, (2) the fact that the disease was limited to infants in the neonatal period, (3) the high mortality rate, and (4) failure to find an etiologic agent by routine bacteriologic methods. Data obtained in more recent years, however, have shown clearly that this is not the case, and it is now quite clear that epidemics of diarrhea among newborn infants may be due to a number of organisms. Gordon and Rubenstein,[2] in 1950, in a reveiw of the subject of "epidemic diarrhea of the newborn," drew the following conclusions on the basis of their study of more than 40 epidemics of diarrhea:

(1) A number of epidemics among the newborn have been shown to be due to *Salmonella* and *Shigella* organisms, but a large number of outbreaks cannot be attributed to these organisms.

(2) The *Shigella* group rarely causes disease among newborn infants. It was not found as a cause of an epidemic in any of the 40 nursery outbreaks studied. The search of the literature made by Gordon and Rubenstein revealed only three epidemics considered to have been caused by *Shigella*.[3-5] One report,[6] however, was overlooked by the authors, which makes, in all, four epidemics of diarrhea in the newborn caused by *Shigella*.

Various members of the *Salmonella* group have caused epidemics of diarrhea among the newborn.[5] These include *S. typhimurium*, *S. wichita*, *S. choleraesuis*, and *S. oranienberg*, the last of which has been reported as causing a recent epidemic of diarrhea in the newborn.[7] It is clear that *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*) also has caused epidemics of diarrhea among the newborn.

The three bacterial species listed above are undoubtedly enteropathogenic, but it is important to realize that they have been isolated only a relatively few times in the hundreds of outbreaks studied. Experience obtained since 1944 (after typing of *Escherichia coli* became possible) makes it clear that the great majority of outbreaks of diarrhea in nurseries for the newborn are caused by enteropathogenic *E. coli*.[8-12] The possibility that infantile diarrhea might be caused by viruses, however, has received considerable attention. There are a number of natural diarrheal diseases among animals that are caused by viruses. For example, a small virus has been isolated that causes a disease of cats termed infectious feline agranulocytosis and feline enteritis.[13, 14] This virus causes leukopenia with very severe neutropenia, vomiting, diarrhea, and nasal and ocular discharges. It is infectious when placed in the stomach. Its pathogenicity is limited to the feline species. Two viruses responsible for diarrhea among suckling laboratory mice have been described. One of these produces intranuclear inclusions in the cells of the intestinal mucosa.[15] Infection with the second virus causes intracytoplasmic inclusions. This naturally-occurring disease has been reproduced experimentally by feeding suckling mice with bacteria-free suspensions of the intestines of infected animals.[16, 17] A disease of calves characterized by enteritis and pneumonitis was shown by Baker to be caused by a virus.[18] Doyle and Hutchings[19] described epidemics of severe diarrhea among baby pigs, and produced the disease in suckling pigs by feeding bacteria-free filtrates of triturates of the gastrointestinal tract and its contents. It should be noted also that distemper virus infection in puppies is often accompanied by diarrhea.

In 1942 Light and Hodes[20, 21] isolated a filtrable virus from newborn infants with diarrhea in 4 separate nursery outbreaks in Baltimore, Md., and Washington, D. C. All 4 virus strains were identical. The virus caused bloody diarrhea in young calves when it was instilled into the nose and throat. Cross-immunity tests showed the virus to be different from the agent that causes calf scours (now known to be due to *E. coli*). Virus-neutralizing serum antibodies developed in infants recovering from the disease, and the same was true of experimentally infected calves. The virus was not isolated from normal calves or from normal babies. It was found to be very resistant to heat; a temperature above 70°C. was required to inactivate it. In this respect, this virus is similar to the viruses of infectious hepatitis and serum hepatitis. Cummings, in Michigan, isolated what appears to be the same virus, or a closely related one, from stools of newborn infants ill with diarrhea.[22] Light and Hodes considered the virus they isolated to be one of the causes of diarrhea among the newborn. They did not believe it to be the sole agent responsible for nursery epidemics of diarrhea, however, and the author holds the same view at the present time.

In 1944 Buddingh and Dodd[23] described a disease of infants and young children with clinical features that included stomatitis and diarrhea; occasionally adults were also affected. A filtrable virus was isolated from the mouth washings and stools of sick infants that caused an inflammatory reaction when placed upon the scarified cornea of rabbits. Serial passage with rabbit eyes was readily obtained. Neutralizing antibody was demonstrated in the sera of convalescents. In one experiment two rabbits became ill with diarrhea after they had been fed suspensions of infected rabbit cornea. Attempts to transmit diarrhea, however, by feeding the intestinal contents of these animals to other rabbits failed. Meiklejohn,[24] in California, obtained positive corneal reactions with the stools of 37 of 45 infants ill with diarrhea. The stools of 5 of 35 normal controls, however, produced a similar reaction. He made many attempts to show neutralizing antibodies in the serum of the sick infants during convalescence. Some of these attempts were successful, but most were not, and for this reason it could not be claimed that the cause of the diarrheal disease under study had been isolated. The fact that some control material causes the same type of reaction as that from sick infants, and the failure to cause diarrhea in animals, casts some doubt upon the significance of the virus described by Buddingh and Dodd.

In 1948 Verlinde[25] isolated a virus from the stools of young infants. This agent was pathogenic for young ferrets. This work, so far as is known to the author, has not been confirmed, but Verlinde's report is quite convincing.

In 1949, during an investigation of an outbreak of diarrhea among premature infants in a nursery for the newborn, my associates in this work and I isolated a previously undescribed virus that produces fatal encephalitis in suckling mice.[26] It cannot be determined whether this virus (that we designated as "Z" virus) came from the stools of the affected children or from the mice under experiment. Serum from the sick infants was not available for testing, and the virus is not neutralized by pooled human gamma globulin. Two adults out of 15 studied, however, showed a high titer against the "Z" virus. On the other hand, Dalldorf[27] has stated that the "Z" virus closely resembles the encephalo-hepatitis virus of mice (JHM strain). The significance of the "Z" virus in the problem of infantile diarrhea does not appear to be great at the moment, but further experience is required before a final opinion can be given.

Since the introduction of the newer tissue culture methods by Enders and his co-workers, thousands of stools have been cultured by these techniques. The development by Enders[28] of relatively simple and effective methods for isolation of viruses from feces has led to the isolation of a great number of viruses that are cytopathogenic in tissue culture. The relation of these viruses to human disease, however, is not known. Two groups of such agents are the adenoidopharyngeal conjunctival viruses (APC viruses) and the orphan viruses (ECHO viruses—enteric cytopathogenic human orphan viruses). Six types of APC viruses have been identified, but only types 3 and 4 have been shown to produce human disease. It has been shown by Huebner[29] and his co-workers that neutralizing antibodies (evidence of infection) against types 1 and 2 APC viruses are present in the blood of a high percentage of young infants. No disease entity has been correlated with the presence of APC virus types 1 and 2. It is therefore suggested that an attempt be made to do so in relation to epidemics of diarrhea among young infants, including the newborn.

The ECHO group of viruses (13 serologic types) has been isolated from some patients with the aseptic meningitis syndrome, resembling nonparalytic poliomyelitis. These viruses have not been connected with outbreaks of diarrhea. Sabin[30] has recently stated that he has isolated a virus, partially related antigenically to ECHO virus type 10, that he believes to be the cause of diarrhea affecting a family of two children and two adults.

In considering possible viral causes of diarrhea in a nursery for the newborn it should be noted that hepatitis of the newborn is often characterized by diarrhea, which may overshadow symptoms of liver damage. Clinical evidence suggests that at least some cases of hepatitis of the newborn may not be due to viruses of serum or infectious hepatitis, but to an unidentified virus. Studies along this line should be carried out by the virologist whenever an outbreak of hepatitis among the newborn is discovered.

In conclusion, it is apparent that most outbreaks of infantile diarrhea are due to enteropathogenic *E. coli*. Evidence has been presented, however, that indicates that one or more viruses have caused epidemics of diarrhea among newborn infants, and that continued search for other viruses capable of causing infantile diarrhea is warranted.

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VIRUS DIARRHEAS OF ADULTS AND THEIR POSSIBLE RELATIONSHIPS TO INFANTILE DIARRHEA

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Knowledge of relationships between the virus diarrheas of adults and those of infants comes chiefly from epidemiologic and clinical observations. Exploration of the virus population of the intestine by tissue-culture methods is proceeding rapidly,[1] and enlightening facts may soon be found. The viruses that we know the most about at present, however, seem to be unimportant causes of diarrhea, while information concerning the viruses or viruslike agents that frequently cause diarrhea is only fragmentary.

Elsewhere in this monograph, Hodes[2] and Sabin[3] respectively discuss 2 different viruses isolated from outbreaks of diarrhea in infants or children, hence these viruses will not be reviewed extensively here. The poliomyelitis and Coxsackie viruses sometimes cause diarrhea (TABLE 1) but this occurs in a relatively small proportion of persons whose intestines harbor these viruses, since these organisms often cause silent infections. The agents that induce the other diseases listed appear to be primary incitants of diarrhea but have never been adapted to laboratory hosts. As mentioned, therefore, this survey consists largely of clinical and epidemiological inferences.

A good point of departure is to array the facts learned about infectious nonbacterial gastroenteritis from experiments in human volunteers. The first volunteer experiments were done by Reimann and his colleagues in Philadelphia.[4] The syndrome they studied is typical of what we now call the afebrile type[5] of the disease. The cardinal features were watery diarrhea, nausea and vomiting, anorexia, and abdominal pain, with little or no fever. In a single experiment, this syndrome was reproduced in subjects who inhaled ultrafiltered pharyngeal washings or fecal suspensions, but not in those who swallowed the same or similar inocula. The disease could not be transferred, however, to other groups of volunteers.[6]

Whether this entity might have been due to a different agent or strain than the Marcy strain,[7] the next to be investigated, has been discussed elsewhere.[8] The Marcy strain can be passaged serially by oral administration of bacteria-free fecal filtrates. Eight consecutive experimental transfers with bacteria-free inocula have been done.[9] In contrast to the Philadelphia agent, pharyngeal washings failed to induce illness whether administered orally or by inhalation. These findings are in full agreement with the results of two separate groups of Japanese investigators who used initial inocula from outbreaks that occurred in different places and at different times.[10-12] In both the Japanese experiments and in ours,[5, 7-9] volunteers fed control fecal inocula of various types have remained well.

The transmissibility of the disease shows that it is an infection. This finding is supported by the outcome of experiments to demonstrate active immunity.

TABLE 1
PRIMARY VIRUS DIARRHEAS*

Causative agents	Frequency of diarrhea in cases	
	Adults	Infants or children
Well-studied viruses		
Poliomyelitis viruses	often	often
Coxsackie viruses A and B	occasional	occasional
Incompletely studied agents		
Infectious hepatitis (hepatitis A)	often	regular
Afebrile nonbacterial gastroenteritis	cardinal sign	cardinal sign
Epidemic nausea and vomiting ("winter vomiting disease")	often	often
Virus causing diarrhea in calves	not known; see Hodes[2]	cardinal sign; see Hodes[2]
Recently classified agents		
Enteric cytopathogenic human orphan (ECHO) viruses[1]	see Sabin[3]	see Sabin[3]

* The primary virus diarrheas are considered to be those apparently associated with propagation of the virus in the intestine and the presence of infectious virus in the feces. The secondary virus diarrheas are considered to be those associated with infection elsewhere than in the gastrointestinal tract.

Subjects recovered from a bout of experimentally induced gastroenteritis show no evidence of recurrence when subsequently challenged with infectious inocula,[8] and we have recently found that the immunity lasts for a year or longer.[13] The result of a cross-immunity test suggests that one or more of the Japanese strains are antigenically similar to or identical with the Marcy strain.[12]

Experience with the Marcy strain has led us to consider watery diarrhea a prerequisite for unequivocal diagnosis of the experimental disease.[13] In natural outbreaks of nonbacterial gastroenteritis, however, diarrhea afflicts a variable proportion of patients. There are epidemics in which it occurs in a minority of cases or hardly at all.[9, 14, 15] This is so in epidemic nausea and vomiting,[16, 17] or "the winter vomiting disease,"[18] and also in another clinical type, for which the term "febrile nonbacterial gastroenteritis" has been proposed.[5]

While such variations in syndromes do fall into recognizable patterns in outbreaks, we may not impute different etiologies to the various entities on the basis of clinical distinctions alone. There is immunologic as well as clinical evidence, however, that the afebrile (Marcy) and febrile (FS) types are caused by different agents. Subjects recovered from one type are susceptible to the other, and there are clear differences in incubation periods and in the course of experimental infections.[5] Unfortunately, attempts to transmit other varieties of nonbacterial gastroenteritis, including such disorders as winter vomiting disease that are manifested chiefly by upper gastrointestinal distress, have not been successful[5, 8, 9] or have not been made.

There are no differential clinical laboratory tests.[19] Under the circumstances, diagnosis cannot be precise. Hence epidemiologic data, while useful, are crude. Nonbacterial gastroenteritis is the remaining disease after known pathogens causing gastroenteritis have been identified. It is more often diarrheal than nondiarrheal, ranks second only to acute undifferentiated respiratory disease in the Cleveland Family Study,[20] and recurs yearly in the families and community.[21] The latter fact, corroborated by other and less complete observations, is an additional reason for assuming that more than one antigenically distinct agent is responsible, since immunity to the Marcy type, as was mentioned, persists for over a year.[13]

Neutralization tests have been done with preinoculation- and convalescent-phase sera of 12 volunteers recovered respectively from the afebrile (Marcy) and febrile (FS) types or both, to determine whether there were increases in antibody to the so-called "ARD-APC" viruses[22, 23] or the "HE" viruses.[24] Types 1 through 6 of the "RI family" (the inclusive term proposed for the ARD and APC agents[25] were tested in HeLa cell cultures, and types 1 through 5 of the HE viruses were studied in both monkey kidney and HeLa cultures. While many of the 12 volunteers had antibody to one or more of these agents, the titers were the same in both serum specimens.[9] It was concluded that past experience with the RI and HE viruses must have been fairly common among the volunteers, as in other populations, and that the Marcy and FS agents are probably distinct from them. This is of particular interest because Sabin[3] has found strains antigenically related to type 4 HE virus in a family outbreak of diarrhea involving both adult and child. The virus isolated by Light and Hodes[26] during infantile diarrhea outbreaks, however, has not been employed in tests with sera from adult nonbacterial gastroenteritis patients.

Some of the HE agents are prototypes in the so-called "ECHO" group of viruses[1] which, by definition, propagate in and cause cytopathogenic effects in human or monkey tissues. The facts that the Marcy agent has failed to manifest itself by cytopathogenic effects in a variety of human tissues or cells or in monkey kidney cultures, and that volunteers fed such cultures remain well, of course support the deduction that it is not related to the ECHO viruses. Adaptation of the methods used to isolate the ECHO viruses is obviously worth trying, however, in infantile diarrhea epidemics. We have studied one small outbreak in this manner.

Diarrhea of the newborn began in a Troy, N. Y., hospital in March 1954, and by August there had been a total of 18 definite and 13 suspected cases. Nine infants died. Although a large number of stool and throat cultures were made and no *Shigella* or *Salmonella* were found, serotyping for *Escherichia coli* was done only on the stools of the last two patients, from which *E. coli* O127:B8 was isolated. This was considered the probable causative agent. The nursery was closed and measures taken to avoid further infection.

On December 28, 1954, eight days after the nursery was reopened, there was another case of infant diarrhea. Three definite and 7 suspected cases developed between that time and January 24, 1955. There were no deaths and no secondary cases in the staff. The negative results of cultures from infants and attending personnel led us to believe that the second outbreak was not one of *E. coli* diarrhea. HeLa cell, human-intestine cell, and monkey-kidney tissue cultures were inoculated with frozen stool specimens (TABLE 2). Three serial transfers were carried out in each passage line with each specimen, but no virus was detected.

While pathogenic serotypes of *E. coli* have been found in association with

TABLE 2

TISSUE CULTURE STUDY OF NONBACTERIAL GASTROENTERITIS IN INFANTS (Troy, N. Y., January 1955)

Patients		Tissue culture results*		
Clinical status	Number	HeLa	Human intestine	Monkey kidney
Diarrheal case	1	0	0	0
Suspect case (loose stools)	1	0	0	0
Suspect cases (watery rings)	5	0	0	0
Contact; no illness	1	0	0	0

* Expressed as the cytopathogenic effect observed during each of three consecutive passages in each type of tissue culture. The original inocula were fecal specimens that had been stored in a CO_2 box.

sporadic diarrhea in adults,[27] outbreaks of *E. coli* diarrhea are not difficult to differentiate from afebrile nonbacterial gastroenteritis by epidemiologic as well as cultural criteria. Adults seem to be much less susceptible than infants to pathogenic *E. coli*.[28] In contrast, the afebrile nonbacterial gastroenteritis agents seem to evoke profuse diarrhea impartially in all age groups. Nonbacterial gastroenteritis is therefore a probable diagnosis when there is a concurrent high attack rate of afebrile diarrhea among adults during an infantile diarrhea outbreak, or when there is secondary spread outside the nursery.[29, 30] In one such epidemic in a maternity hospital the attack rate in mothers and staff was approximately double the rate in infants.[31] The provisional diagnosis of nonbacterial or "virus" gastroenteritis is still warranted even though the epidemic occurred before *E. coli* serotyping was employed.

Several other diseases should be mentioned. So-called "'epidemic' colic with steatorrhea" has been described in infants and children.[32] Steatorrhea may be a result of virus infection.[3] Infectious hepatitis seems frequently to be a diarrheal syndrome without jaundice in infants.[33] It characteristically runs a prolonged course, which helps in diagnosis. This disease is an example of a virus infection in which diarrhea is more prominent in the young than in adults. The same might also be true of the febrile type of nonbacterial gastroenteritis due to the FS strain.[5] In adults this syndrome is usually nondiarrheal, but it might be diarrheal in infants. These relationships are not now known, but will certainly be defined by experiment once the causative viruses have been isolated.

Summary

Important causative agents of adult diarrhea have not yet been adapted to laboratory hosts; that they are viruses is unproved. Viruses isolated during investigations of infantile diarrhea outbreaks have been studied only to a limited extent, and knowledge of their relation to adult diarrheal diseases is fragmentary. Knowledge of relationships between virus diarrheas of infants and adults therefore comes chiefly from epidemiologic and clinical investigations.

Afebrile nonbacterial gastroenteritis is a widespread diarrhea that afflicts all age groups. An attack confers immunity. At present only one type has been detected in the United States and Japan, but the number of trials has been limited. From the correlation of immunity studies with epidemiologic data, it is inferred that there might be a family of such agents.

Relationships with other viruses have been studied by testing active immunity of volunteers or by neutralization tests of their sera with known viruses. No relations with the so-called RI or HE viruses have been found by neutralization tests up to the present time.

Differentiation of nonbacterial gastroenteritis from *E. coli* diarrhea is aided both by laboratory tests and epidemiologic data. There seems to be a relatively low concurrent attack rate among adults in nursery outbreaks of diarrhea due to pathogenic *E. coli*, and a high one in nonbacterial gastroenteritis. Nursery outbreaks of infantile "virus" diarrhea may not involve adults.

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THE SIGNIFICANCE OF VIRUSES RECOVERED FROM THE INTESTINAL TRACTS OF HEALTHY INFANTS AND CHILDREN

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The viruses that may be recovered from the alimentary tract (throat and stools) of healthy infants and children vary with the method used for their detection, and they fall into two main categories: (1) those known to be the cause of specific diseases, *e.g.* poliomyelitis, herpangina (Coxsackie A), epidemic pleurodynia (Coxsackie B), the pharyngeal-conjunctival syndrome (APC), herpes simplex, and presumably also those of infectious hepatitis, mumps and influenza B; (2) the large number of recently recognized viruses whose relationship to various disease syndromes is still a matter of current and future investigations. The newborn-mouse technique yields predominantly members of the Coxsackie group and also, less frequently, the herpex simplex virus. Rhesus or cynomolgus monkey-kidney tissue cultures most frequently yield the new group of many antigenically distinct "orphan"[1] or HE (human enteric)[2] viruses recently established by committee action as the ECHO (enteric cytopathogenic human orphan) group of viruses[3] and, less frequently, the poliomyelitis and certain other viruses. Certain members of the Coxsackie group (particularly A9 and group B) and of the new "APC"[4] (adenoidal-pharyngeal-conjunctival) or RI group[5] of viruses, as well as the herpes simplex, mumps, and influenza B viruses can also be detected by monkey-kidney cultures. Most of the APC viruses and certain types of Coxsackie virus, however, are more readily detected in cultures of HeLa cells, while many of the ECHO viruses multiply poorly in HeLa cells and may not be readily recovered in this type of culture medium. It is highly probable that the use of additional cell lines in tissue-culture media will reveal still more hitherto-unknown viruses.

It is the classic tradition of man's quest for knowledge about himself and his environment that we should want to learn as much as possible about the seemingly bewildering number of new viruses that appear to be part of the human heritage. I should like to begin by summarizing what my associate M. Ramos-Alvarez and I have learned during the past 2 years by testing rectal swabs from 3347 healthy children in cynomolgus monkey kidney tissue cultures. From 1566 healthy children aged 1 to 17 years tested in the region of Cincinnati, Ohio, during the months of July and August 1953, we recovered 31 strains of virus—5 were poliomyelitis, 1 a Coxsackie B4, and the remaining 25 constituted 5 new antigenic types of what we first called HE viruses, types 1 to 5, and that will now be known as the ECHO viruses types 7 to 11. From 1771 healthy children aged 1 to 4 years tested in Mexico City and Veracruz, Mexico, during early June, 1954, we recovered 334 strains of virus, of which 73 were poliomyelitis viruses and 261 were other viruses requiring classification. It should be pointed out that in all 3 areas, the children came from the low socioeconomic groups, that they had no known contact with clinically recognized cases of poliomyelitis, and that there was no unusual incidence of poliomyelitis in the community at the time.

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An analysis of the Cincinnati data revealed that the carrier rate for the ECHO viruses diminished with increasing age. It was 5.2 per cent among 154 in the group aged 1 to 4 years, 2.6 per cent among 537 in the group aged 5 to 9 years, 0.2 per cent among 683 in the group aged 10 to 14 years, and none was recovered from 154 adolescents aged 15 to 17 years. Similar but less striking was the drop in the carrier rate of poliomyelitis viruses from 0.6 per cent in the group aged 1 to 4 years to 0.2 per cent in the group aged 10 to 14 years. By comparison, it is noteworthy that the carrier rate among the children aged 1 to 4 years in Mexico was as follows:

City	No. tested	Carrier rate (per cent) for	
		Polio	Other viruses
Mexico City	1491	3.4	15.6
Veracruz	280	8.2	10.0

When the Mexican data are analyzed by individual years, one observes a similar drop in the carrier rate for the poliomyelitis viruses from about 4 per cent at age 1 and 2 years to 1.7 per cent at age 4 in Mexico City, and from about 12 per cent at age 1 and 2 years to 5.8 per cent at age 4 in Veracruz. As regards the nonpoliomyelitis viruses, however, the carrier rate was about the same for each of the 4 ages in Mexico City, but less among the 4-year-old children in Veracruz. With the great multiplicity of antigenic types among the ECHO viruses, it is conceivable that even under conditions most conducive to fecal spread, many years may pass before any one individual may have an opportunity to become infected with all of them. A serologic survey carried out with the 5 Cincinnati HE viruses (ECHO types 7 to 11) on 31 sera from Cincinnati children aged 1 to 5 years and on 30 medical students aged 20 to 30 years revealed the presence of antibody against all 5 viruses in both age groups, the incidence of positives in all instances being higher among the older individuals. It is noteworthy, however, that although 87 per cent of the young adults had antibody for one or more of these 5 types of viruses, only 3 per cent had antibody for all 5. Serologic tests on sera from 19 children aged 1 to 4 years from Jamshedpur, India (kindly sent to us by Max Theiler of the Rockefeller Foundation, New York, N. Y.) indicated not only that all 5 Cincinnati ECHO viruses occur in India but also that the incidence of infection was as high in the very young children in India as among the medical students in Cincinnati.

Four of the 5 Cincinnati viruses (HE 2, 3, 4 and 5 or ECHO types 8 to 11) were found among the 233 nonpoliomyelitis viruses recovered from healthy children in Mexico City (27.4 per cent of the total), and only one of the Cincinnati types (HE5) was found among the 28 Veracruz strains (7 per cent of the total). The Cincinnati HE1 virus (ECHO type 7), which constituted 32 per cent of the 25 Cincinnati strains, was not found among the 261 Mexican strains.

It is obvious that antigenic classification constitutes the first task in the systematic study of this new group of viruses. J. L. Melnick and his associates had already segregated a number of antigenically distinct prototypes from among a large number of "orphan" viruses recovered from patients with suspected poliomyelitis, and W. McD. Hammon and his associates had segregated several distinct prototypes from among a large number of strains recovered from healthy children in the Philippine Republic. A comparative study of the prototype strains available in the New Haven, Conn., Pittsburgh, Pa., and Cincinnati, Ohio, laboratories led to the establishment of the first 13 antigenically distinct members of the ECHO group of viruses. That these 13 types represent only a fraction of the viruses that have already been recovered from the intestinal tracts of healthy children is evident from an analysis just completed by my associate, M. Ramos-Alvarez on 142 of the 195 Mexican nonpoliomyelitis strains that remained unclassified after tests with the 5 Cincinnati HE prototype antisera (ECHO types 7 to 11). Tests were carried out with pools of the 8 remaining ECHO antisera (using sera kindly supplied by Melnick and Hammon) and of 6 Coxsackie antisera (A9, A14 and B1 to B4—kindly supplied by Syverton). Only 11 per cent of the 142 strains could be classified with these pools—6 strains proved to be Coxsackie viruses (three A9 and one each of A14, B2, and B3) and 10 belonged to the other ECHO viruses. With only a few exceptions, the large number of remaining strains produce a poliomyelitislike cytopathogenic effect in tissue culture. Further classification will be achieved only by the laborious preparation of antisera and successive sorting out of the remaining strains. This task will be greatly facilitated if all investigators who might engage in similar work would use the Committee on ECHO Viruses recently established by The National Foundation for Infantile Paralysis, New York, N. Y. as a clearinghouse for newly established prototypes.

In the meantime, what can one say about the significance of this large number of new viruses that have been found to inhabit the human alimentary tract? It is already evident that they are not the viral counterpart of the "normal" bacterial flora, because they are most frequently recovered during the early years of life and only rarely thereafter. Repeated tests on the stools of 80 human adults who served as volunteers in experimental studies with attenuated poliomyelitis viruses in no instance yielded an ECHO virus. The large number of ECHO viruses that have been recovered from patients with the aseptic meningitis syndrome and the fact that some of those recovered from healthy children have also been encountered in patients with this syndrome emphasizes the need for future studies on the etiologic relationship of certain ECHO viruses to this syndrome. The possible role of some ECHO viruses in infections of both the respiratory and enteric tracts is emphasized by two recent observations in my laboratory that will be reported in greater detail elsewhere: In one instance an epidemic of rhinitis in chimpanzees was shown to be caused by a virus recovered from the stools of sick chimpanzees in cynomolgus monkey-kidney tissue cultures. This virus reproduces rhinitis in chimpanzees and is antigenically related to but distinct from the Cincinnati HE4 or ECHO type 10 virus derived from the stools of healthy children. In another instance, a very similar antigenically related virus was recovered during the course of a family epidemic of a "steatorrheic enteritis" that was characterized by lower-abdominal pain and frequent fatty stools. The course was afebrile and mild in the parents and 1 older child but febrile and relatively severe in the 3-year-old child from whose stools the virus was recovered in amounts of at least 10^8 TCD_{50} per gram on two occasions. The parents and both children developed antibody for this virus during convalescence.

Of particular interest to the conference on which this monograph is based was the question of the possible role of some of the ECHO viruses as etiological agents of the epidemic and endemic diarrheal diseases of early childhood. During the past summer M. Ramos-Alvarez initiated such a study on infants and children admitted to the Cincinnati General Hospital and the Children's Hospital, Cincinnati, with diarrhea as the chief complaint. Rectal swabs and acute and convalescent phase sera were obtained for study. The following preliminary results of the tests in monkey-kidney tissue culture give some indication of the nature of the problem. The rectal swabs from 56 children under 4 years of age (38 under 1 year of age) yielded 24 viruses (43 per cent positive). It is highly probable that many more strains will be recovered when the swabs, which yielded negative results in cynomolgus monkey-kidney tissue culture, are tested in HeLa cells and human amniotic tissue cultures, as well as in suckling mice. Among the 24 recovered viruses, 3 were definitely type 1 poliomyelitis, suggesting that diarrhea may be part of the poliomyelitis syndrome in infancy, while constipation is more common in older children and adults. Three strains turned out to be Coxsackie viruses (one A9 and two B2), 2 were classified among the 13 known ECHO viruses (one type 2 and one type 8), and 12 strains could not be identified with any of the known ECHO viruses. Among 17 patients who yielded viruses, bacteriological studies yielded *Shigella flexneri* in 2, *Salmonella paratyphi* C in 1, *Proteus mirabilis* in 2, *Proteus vulgaris* in 1 and a paracolon bacillus in 1. The high incidence of positive virus isolations among infants and children with diarrhea warrants a continuation of such studies until enough information has been accumulated to permit a sorting out of viruses and associated clinical manifestations. The potential importance of certain APC viruses among the syndromes with diarrhea is emphasized by a recent observation of Svedmyr[6] on a type 3 outbreak in Kumla, Sweden, in which 40 per cent of the laboratory-confirmed cases exhibited diarrhea.

Summary

In addition to the viruses previously recognized in the human intestinal contents, the use of monkey-kidney tissue cultures has revealed a new, large, and heterogeneous group of many antigenically distinct viruses recently established as the ECHO (enteric cytopathogenic human orphan) group. These viruses occur with great frequency in very young children and only rarely in adults, and are therefore not the viral counterpart of the normal bacterial flora. The role of the poliomyelitis, Coxsackie, APC and ECHO viruses in various syndromes in which diarrhea may be a prominent clinical manifestation can now be investigated by suitable laboratory methods.

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